VOL. LVIII, NO. 659 NOVEMBER-DECEMBER, 1924

THE AMERICAN NATURALIST

A BI-MONTHLY JOURNAL

Devoted to the Advancement of the Biological Sciences with Special Reference to the Factors of Evolution

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THE SCIENCE PRESS

LANCASTER, PA. GARRISON, N. Y.

GRAND CENTRAL TERMINAL, NEW YORK CITY

The American Naturalist

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THE SCIENCE PRESS

Lancaster, Pa. New York, N. Y. Garrison, N. Y.

Entered as second-class Matter, March 19, 1908, at the Post Office at Lancaster Pa., under the Act of Congress of March 8, 1879.

THE
AMERICAN NATURALIST

VOL. LVIII *November-December, 1924* NO. 659

THE STABILITY OF SUBSPECIFIC CHARACTERS UNDER CHANGED CONDITIONS OF ENVIRONMENT

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INTRODUCTION

IN various recent papers I have discussed the geographic variation of mammals and birds, and particularly the marked correlation which exists between certain characters of local races and features of their physical environment. While this last fact has long been recognized by naturalists, and has been the subject of considerable theoretical discussion, the problems here raised have rarely been subjected to experimental test.

Not many years before the present studies were commenced, it was possible for Jordan to write:

> It remains, however, to be determined whether these environmental forms —these species and subspecies produced by the direct influence of heat, cold, humidity and aridity—are "ontogenetic species" . . . or whether they have a real existence outside the lifetime of the individuals actually composing the group or species. We do not know which of the traits induced by direct action of the environment, if any, are actually hereditary and which are not.

Referring to certain possible experiments with woodpeckers, Jordan argues that if the parental color tone were retained in the progeny of a stock which was transferred from Vancouver to Arizona, "then humidity would be a real factor in the formation of species." If, on the other hand, the transplanted birds "should develop in the fashion of the local race of this region . . . then the duskiness is not a true specific or subspecific character. . . . It may be that these questions have been

already solved by experiments on birds, but if so, the experiments have escaped my attention."

The writer thereupon suggests some profitable undertakings with birds, remarking in this connection: "Perhaps our ornithologists will some day test their species and subspecies by a test of the permanence of this class of characters.[1]

In a reply to this significant utterance by Jordan, J. A. Allen lays stress upon the fact "that the local differentiation in color between the subspecific forms of a given group is often (but not always) much more strongly expressed in the first pelage or plumage of the young than in the adults of the same forms." This he regards as sufficient evidence "that local differentiations are transmitted from parent to young, and are hereditary in the usual sense of that term." He continues:

> Doubtless no one questions their continued transmission from generation to generation so long as the environment remains stable. Probably also few would question that were representatives of a strongly marked local form . . . to be transplanted to a region markedly different climatically from their natural home, they would gradually lose their original characteristics and become, after a number of generations, more or less modified, in better agreement with the new conditions of life. But it would be apparently rash to expect a very material change in a single generation.[2]

Allen is, however, unable to offer any exact positive evidence on the subject unless we except the single case of a quail which had been introduced (presumably) from Florida into Cuba, and which, after a hundred years, was said still to be "impossible to distinguish from the Florida bird," thus differing "in a marked degree" from the Cuban form.

These statements by Jordan and Allen bear testimony to the lack of critical evidence in this field as late as 1906.[3] Even at the present day, indeed, doubts are occasionally expressed as to the fixity of subspecific characters, particularly among birds. Thus only three years

[1] *Science*, December 29, 1905.

[2] *Science*, January 26, 1906.

[3] Vaughan (*Science*, May 4, 1906), in this same year, asks some pertinent questions of a similar nature to Jordan's.

ago, Lowe and Mackworth-Praed[4] expressed themselves as follows:

> We think there can be little doubt that many—indeed, by far the majority—of our present-day subspecific forms belong to this last category ["fluctuational" and non-heritable, as contrasted with "mutational"] and are mere environmental, unstable and essentially superficial variations, which would quickly disappear if the organism were transferred from its normal environment to some other of a different nature.

Of the various interrelated questions which demanded solution, the first and most obvious, of course, was whether these subspecific differences were inherited at all. Would they persist *to any extent* if two different geographic races were reared in a common environment, or might not all subspecies prove to be purely "ontogenetic," in the sense in which Jordan (following Kellogg) employed that term?

At the close of 1913, opportunity was offered the present writer by the Scripps Institution for Biological Research to put this question to an experimental test. The most abundant, variable and widely distributed species of deer-mouse, *Peromyscus maniculatus,* was chosen as the most promising material for this investigation. In the spring of 1914, I trapped a preliminary series of living *P. m. sonoriensis,* in the Mojave Desert, near Victorville, California, and took these to Berkeley for breeding purposes. In August, 1915, I was able to report that "*neither the originally introduced animals nor their offspring, nor their grandchildren, have thus far shown any perceptible approach to the local type* [*gambeli*]."[5]

In 1915, the *Peromyscus* studies were transferred to La Jolla. The material employed in the transplantation experiment now included specimens of a dark race, *P. m. rubidus,* from the redwood forests of Humboldt County, California, as well as a fresh series of *sonoriensis* from Victorville. In 1917, it was possible to state, for both of these subspecies, that no evidence of color change had

[4] *Ibid,* April, 1921 (this article was prompted by one by L. M. Loomis, expressing a similar viewpoint).

[5] AMERICAN NATURALIST, November, 1915.

been detected, although they had been bred as far as the third cage-born generation.[6]

This was not true, however, of some other characters. It early became evident that cage-bred mice, belonging to all these geographic races, tended to be stunted more or less by some unknown influence pertaining to the conditions of captivity. This stunting affected the relative length of certain appendages, as well as the size of the body as a whole, and thus led to the modification of some of the very characters which are of most importance in the comparison of subspecies. It was evident, however, that the local race, *gambeli,* was modified in the same direction as the two introduced races, *rubidus* and *sonoriensis.* The measurement of considerable series, belonging to each of these subspecies, showed that the relative positions of these three races were but slightly affected by the changes in question. There was found, it is true, some degree of convergence on the part of *rubidus* toward *gambeli.* But the fact that *sonoriensis* was found to *diverge more widely* from *gambeli* in the C_2[7] generation than in the parent generation deprived the former fact of all significance.[8] That there is, in general, no tendency toward a convergence of the introduced types will be shown later.

The results of these simple experiments made it plain that for one species of *Peromyscus,* at least, subspecific differences are to a large extent hereditary. Without going further, the evidence was sufficient to refute the supposition that these differences are wholly, or even chiefly, the result of climatic or other conditions, acting within the lifetime of the individual.

There remained, however, the important question of the degree of stability of these subspecific characters.

[6] Bulletin of the Scripps Institution, No. 3, October 19, 1917 (University of California Press).

[7] The various cage-born generations have been designated C_1, C_2, etc.

[8] AMERICAN NATURALIST, June–July, 1918, p. 292. The pathological nature of the changes in this *rubidus* stock was likewise shown by the fact that the strain became so largely sterile that it was impossible to continue it further. The behavior of a normal strain will be discussed below.

The data thus far cited are plainly not adequate for the answer of this question. A slight tendency toward convergence on the part of the introduced races might well fail of detection in the absence of sufficient numbers of specimens. Or the effects of the local climatic conditions might be masked by the deformations due to captivity. For the purposes at hand, it is evident that we need larger series of more normal animals, a greater number of generations and more precise quantitative treatment of the material. In the case of the data next to be presented, these conditions have, to a considerable extent, been realized.

Later Transplantation Experiments

Before considering the more precise data from these later experiments, it will be well to discuss briefly some of the climatic differences between the localities here considered. My thanks are due Professor H. H. Collins, now of the University of Pittsburgh, for important assistance in computing the meteorological data here offered.

Victorville is situated near the western border of the Mojave Desert, at an altitude of 2,700 feet, and about twenty miles from the mountain barrier which divides the desert from the coastal plain. La Jolla is situated directly on the ocean shore, about fifteen miles north of San Diego. Carlotta lies near the opposite end of the state, in a district until recently occupied everywhere by dense redwood forests. The region is one of fairly high rainfall, frequent heavy fogs and overcast skies.

In Table I are summarized certain meteorological data for these three regions.[9] There are several salient points revealed by a comparison of these figures: (1) In respect both to atmospheric humidity and to rainfall, the desert station represents the extreme of aridity, and that of the northwest coast the extreme of humidity, with La Jolla occupying an intermediate position. It is probably no mere coincidence that in respect to depth of pigmenta-

[9] Data for Eureka, rather than Carlotta, are given, owing to lack of records for the latter point. The two are about twenty miles apart.

tion, the three local races of *Peromyscus* follow the same order, the desert race being the palest. (2) In respect both to daily and seasonal range, and to that of temperature as well as that of humidity, the figures for the desert are far in excess of those for the northwest coast, La Jolla again being intermediate. (3) In respect to mean annual temperatures, the order is somewhat different, La Jolla and Eureka representing the extremes, while Victorville is intermediate. Although deserts in general are regions with high summer temperatures, this fact is counterbalanced by their relatively low winter temperatures. That the mean temperature of Victorville is actually lower than that of La Jolla is not surprising in view of its greater elevation and higher latitude.

TABLE I

CLIMATOLOGICAL DATA FOR LOCALITIES CONSIDERED IN THE PRESENT PAPER[10]

	Temperature			Relative Humidity			Rainfall
	Annual mean (° C.)	Mean daily range	Seasonal range (difference between highest and lowest monthly mean)	Annual mean	Mean daily range	Seasonal range (difference between highest and lowest monthly mean)	Annual mean
Victorville	14.0	20.4	20.4	49.8	57.0	14.5	6.2
La Jolla	16.3	7.4	10.4	73.8	24.1	11.1	10.0
Eureka	9.8	6.1	8.1	86.7	15.8	9.4	46.0

[10] Temperature and humidity data are based upon records of thermographs and hygrographs, checked by mercury thermometers. Records were kept at Victorville, from December, 1914, to January, 1917, inclusive, the instruments being in charge of Mr. Ralph H. Webb. At Eureka, the records were kept from November, 1914, to January, 1917, inclusive, Mr. B. S. Nichols, of the U. S. Weather Bureau, having charge of the instruments. At both of these stations the instrument shelters were purposely placed much nearer the ground than is done when "standard" meteorological observations are undertaken. At Eureka, the instruments were placed among underbrush, in a strip of redwood forest.

At La Jolla, meteorological instruments were installed in the murarium, almost from the commencement of these experiments. Owing to the construction of this building the temperature and humidity conditions are very

Peromyscus maniculatus sonoriensis: The parent stock of this series was trapped near Victorville, in April, 1915, and was brought at once to La Jolla. For six successive generations these mice were reared in the "murarium,"[11] in our standard laboratory cages, having the dimensions 16 x 9¾ x 9¾ inches. The stunting effects of captivity were manifest, to some extent, even in the C_1 generation, while measurements of the C_2 generation showed that the mice had undergone a considerable reduction in mean body length, as well as in the mean size (both relative and absolute) of some of the measured parts.[12]

Fifteen animals (3 ♂, 12 ♀), belonging to the C_5 generation, and 13 (3 ♂, 10 ♀), belonging to the C_6 generation, were transferred to two of our open pens, August 18, 1919. The pens in question each have an area of about 12½ x 12½ feet. They are covered by two layers of wire screen, and are bounded, beneath the ground, by concrete walls, sunk to such a depth as to prevent burrowing animals from entering or leaving them. The floor of these pens is constituted by natural soil, into which

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nearly the same as in a "shelter" of the usual pattern. Records for three years (September, 1915, to August, 1918) were utilized in obtaining the present humidity data, while only two of these years were used in the case of temperature.

Annual means, both for temperature and humidity, were obtained by averaging the daily means, these last, in each case, being the mean of the daily maximum and minimum. The laborious task of digesting these records and making the necessary computations was performed by Dr. H. H. Collins.

The recording apparatus, though adjusted at times, and checked at weekly intervals by more reliable instruments, was subject to variable and frequently considerable errors. Comparison with Weather Bureau records for Eureka and San Diego reveals, however, a rather unexpected degree of correspondence between our records and the official ones. While doubtless not adequate for exact meteorological studies, the data here given certainly suffice to show the climatic differences between the localities in question.

The figure for rainfall at Victorville is based upon rain-gauge records kept for 11 years by Mr. Reginald Frost. For La Jolla, the figure for San Diego has been employed. The Eureka figure is based upon Weather Bureau records for that station.

[11] This building is so constructed that the outside atmosphere circulates freely through it.

[12] AMERICAN NATURALIST, June–July, 1918, pp. 290–293.

the mice burrow freely. Food is brought them twice weekly.[13]

At the time of this transfer, the C_5 animals were 10 to 11 months old, the C_6 ones being, for the most part, three to four months old. There was considerable stunting and some actual deformity (*e.g.*, curved feet) among the *sonoriensis* stock at this time. It is probable that the more normal individuals were selected for the pens.

It should be superfluous to state that pedigree breeding, such as is practiced habitually in our small cages, is impossible in these large inclosures. Ordinarily, it is not even possible to know the generation to which a given individual belongs.

The *sonoriensis* stock was kept in the pens referred to for a period of nearly four years, in the course of which period the animals were all trapped out twice and part of the stock eliminated. On each of these occasions, the lot which was returned comprised none of the individuals which had been introduced on the previous occasion.[14] Since the original lot of mice which was placed in the pens belonged to the C_5 and C_6 generations, it is evident that the lowest possible number of generations represented by any mouse at the end of the experiment was seven, with a probable minimum of eight generations for half of the stock. It is quite unlikely, however, that these minimum figures indicate fairly the average number of generations which were produced during the period of the experiment. They leave out of account the fact that many of the mice were known to be breeding actively during this period, and that *Peromyscus* sometimes begins to reproduce at the age of 3 or 4 months.

It would be a conservative statement that the specimens of *sonoriensis* which were measured and skinned in October, 1923, represent a minimum of seven generations bred at La Jolla, and a maximum of twelve or more, while the majority probably belonged to the eighth to the tenth generations.

[13] They are not fed daily owing to the distance of these pens from the other buildings of the station.

[14] Recognition of these was possible by means of identification marks.

Fifty-three specimens (18 ♂, 35 ♀) were chosen for measurement. Save that obviously immature individuals and those with damaged pelages were rejected, care was taken that the choice of specimens should be random.

The occupants of these pens are much prone to fighting and inflict many minor injuries upon one another. In particular, the tail and rump regions suffer most from this persecution. Specimens with tails more or less abbreviated are not infrequent, and the measurements of such tails can not, of course, be included in our computations. For this reason, it has been necessary to reject the tail length of 5 *sonoriensis* and 19 *rubidus*.

Injuries to the skin frequently leave lasting effects, even when healing is complete. Thus damaged areas may show pale spots, due to the replacement of normal, colored hairs by white ones. Careful examination reveals the presence of such spotting in a considerable proportion of pelts from the open yard stock, the posterior dorsal region of the skin being most affected. When these traumatic changes are sufficiently pronounced, they appreciably affect the coat-color, and it has been necessary to reject such specimens from our series. The *sonoriensis* stock has suffered much less in this respect than the *rubidus* stock.

For the purposes of the color analysis, a rectangular area of standard size (31 x 17 millimeters) and nearly constant position was subjected to examination in each case. The rectangle chosen was situated transversely, across the posterior dorsal region of the pelage, and a little in advance of the base of the tail. The color determinations were made with the Hess-Ives tint photometer, the use of which instrument for the study of mammalian coat-color has already been discussed in several previous papers.[15]

In Table II are given the means and standard deviations for the four values which have been considered

[15] *Journal of Mammalogy*, May, 1921 (the procedure there given has been somewhat modified); *Journal of Experimental Zoology*, October, 1922; *ibid.*, October, 1923.

throughout these studies. The first line of figures are those for a series of wild specimens of *sonoriensis* which had been trapped at Victorville.[16] This lot includes some of the actual ancestors of the experimental series. In the second line are the figures for this same race, after a residence of more than 8 years at La Jolla, and the lapse of from 7 to (probably) 12 or more generations. All the latter animals and a large majority of the former were killed in the fall (September and October).[17]

TABLE II

COAT-COLOR ANALYSIS OF PEROMYSCUS MANICULATUS SONORIENSIS

	No.	Black		White		Color		Red : Green	
		Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
Wild[18]	49	77.14 ± .21	2.22	11.94 ± .11	1.19	10.91 ± .15	1.53	2.87 ± .02	.22
Transplanted	47	75.69 ± .18	1.86	13.66 ± .12	1.25	10.65 ± .14	1.47	2.81 ± .02	.22

It will be seen that there are small though significant differences between these two series in respect to the proportions of black and white. This corresponds to the

[16] In order to be sure of the full maturity of these specimens, only those were included which had been kept for 4 months or more after capture.

[17] Somewhat more favorable skins would have been obtained had all the animals been killed after the completion of the fall molt. Many skins belonging to both series give evidence of molting on the reverse side, though the color tone of the pelage is seldom materially affected thereby.

Winter pelages appear to average a trifle darker, in this species, than those of the summer and fall, a fact which may account for a small part of the difference between the two series comprised in Table II. The relative values are but slightly changed, however, when the comparison is restricted to fall pelages.

[18] These figures for the "wild" mice differ somewhat from those published in a previous paper (*Journ. Exp. Zool.*, October, 1923) for the same series. This is due to the fact that they represent a later set of readings, made at the same time as those for the "transplanted" series. Owing to the influence of atmospheric conditions upon photometer readings of these skins, strictly comparable figures can not be obtained unless the conditions are identical. It is my practice, when two series are to be compared, to repeat each of the readings on a different day, so alternating the two lots as to distribute equally the effects of any such disturbing factors.

fact that the "transplanted" series, even to the unaided eye, averages somewhat paler than the parent ("wild") lot. The proportions of "free" color are, however, closely alike in the two series, while the spectral position of this color, as indicated crudely by the "red: green ratio," is almost identical in the two.

The reasons for this slight excess of "white" and the corresponding decrease in "black," on the part of the experimental series, can not be stated with any certainty. The difference may have no biological significance whatever. In any case, it must be urged that such a change, if it actually occurred, was not in the direction of the local type, *gambeli,* but quite the reverse. For *P. m. gambeli,* like other residents of the mountains and coastal plain, is distinctly *darker* than the desert race, *sonoriensis.* (See Table V.)

In Table III are given the mean values for linear measurements of the body and some of its appendages, along with those for the relative width of the dorsal tail stripe (ratio to circumference of tail), and the depth of pigmentation of the soles of the feet, graded according to an arbitrary standard.[19]

It will be seen that the "transplanted" series comprises animals having a somewhat greater mean body length than those of the "wild" series. Since the maximum size is the same for the two lots, this is without doubt due to the fact that the latter one includes a certain proportion of immature mice, and does not represent an increase in the size of the captive animals.[21] This mean difference in general size makes it necessary to reduce the measurements to a common standard, before comparing the length of the tail, foot and ear in the two series of mice. In Table IV are given the approximate values which would have been found if all our mice had

[19] *Journal of Experimental Zoology,* April, 1920; October, 1923.

[21] It is my practice, in measuring wild mice, to include animals having a body length of 80 millimeters or more. Under these conditions, it is inevitable that some immature individuals should be included. These smaller specimens are not, however, skinned.

TABLE III

VARIOUS MEASURED CHARACTERS OF P. M. SONORIENSIS (ACTUAL MEANS)[20]

	No.	Body length		Tail length (abs.)		Foot length		Ear length		Tail stripe (%)		Foot pigmentation	
		Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
Wild	140	88.99 ± .24	4.18	72.36 ± .31	5.33	♂ 19.91 ± .04 ♀ 19.58 ± .06	.57 .66	17.17 ± .04	.73	28.12 ± .24	4.11	.90 ± .07	.68
Transplanted	53	90.81 ± .35	3.77	69.36 ± .43	4.43	♂ 19.59 ± .06 ♀ 19.34 ± .08	.37 .68	17.17 ± .07	.68	24.85 ± .30	3.22	.77 ± .06	.60

[20] For certain characters, the number of available individuals was less than the total number indicated, owing to the injury of certain parts in some specimens (see p. 489). This fact accounts for certain minor discrepancies in the magnitude of the probable errors. The number of "wild" mice upon which the figures for foot pigmentation are based is 49, instead of 140. These are the same 49 individuals the skins of which were used in the color tests. Foot measurements are given separately for males and females, owing to the pronounced sexual difference in the length of the foot.

had a body length of 90 millimeters.[22] The figures for the two other characters require no such correction, since these are more nearly independent of general size.

TABLE IV

CORRECTED VALUES OF TAIL, FOOT AND EAR IN P. M. SONORIENSIS

	Tail	Foot (♂)	Foot (♀)	Ear
Wild	73.09 ± .31	19.97 ± .04	19.56 ± .06	17.26 ± .04
Transplanted	68.82 ± .43	19.64 ± .06	19.17 ± .08	17.08 ± .07

From Tables III and IV it appears that the mean values for tail length, foot length (in both sexes) and width of tail stripe are significantly lower in the animals which have been reared for a number of generations at La Jolla. That these differences do not represent a modification of the characters concerned, as a result of changed climatic conditions, is, I think, equally plain. For (1), in each instance, the figure for the "transplanted" series of *sonoriensis* agrees less closely with that for the local race, *gambeli*, than does the figure for the "wild" series (*cf.*, Table V); and (2) the changes are in the direction of those to which all races (including *gambeli*) are subject, as the result of captivity, irrespective of climate.

Peromyscus maniculatus rubidus: Some preliminary observations on an earlier lot of these mice from Eureka have been discussed above. Because of the unsatisfactory nature of these results, a new stock was trapped near Carlotta, Humboldt County, in October, 1917. Carlotta lies about 20 miles from Eureka and is considerably further from the coast. In the wild generation, the Eureka and Carlotta series of mice showed no significant differences, except in the color of the pelage, which was appreciably paler, on the average, in the Carlotta animals. The latter were, nevertheless, much darker than the La Jolla race (*gambeli*).

[22] See *Journal of Experimental Zoology*, April, 1920, p. 385.

TABLE V

MEAN VALUES FOR CERTAIN CHARACTERS IN LA JOLLA RACE OF P. M. GAMBELI (CORRECTED WHERE NECESSARY)[23]

Tail length	75.52 ± .27
Foot, ♂	20.02 ± .06
Foot, ♀	20.13 ± .04
Ear	17.80 ± .04
Tail stripe	32.34 ± .26
Foot pigmentation	1.88 ± .09
Black	83.47 ± .23
White	9.70 ± .13
Color	6.83 ± .16
Red : Green ratio	2.96 ± .04

Carlotta mice of the C_2 generation were transferred to the open pens in August, 1919, the stock having been previously kept in small cages within the "murarium." The treatment, subsequent to this transfer, was the same as that employed in the case of *sonoriensis* (p. 488). In the present case, the mice which were ultimately measured, with a few exceptions, represented a minimum of four generations born at La Jolla, and a maximum of six or more. The exceptions were 7 individuals (out of 62) which belonged to the C_3 generation.[24]

In Table VI are given the color values for this series of "transplanted" mice, along with those of the parent stock from which they were derived. Only fully mature pelages are here included. The skins of the former series were prepared in October, those of the latter in September and October. Here, as in the case of *sonoriensis*, many skins give evidence of molting on the reverse side, although here, too, the appearance of the pelage is seldom materially affected by this circumstance. Of the 62 skins originally prepared, it was necessary to reject 21, owing chiefly to the presence of white hairs, following injury (see p. 489). Indeed, this condition may be detected, to some slight degree, in a large majority of the pelages.

[23] The figures for tail length to tail stripe, inclusive, are based upon 175 wild individuals; those for the remaining characters upon 52 cage-bred (C_3) individuals.

[24] These were used for body measurements, but not for skins.

TABLE VI

COLOR ANALYSIS OF PEROMYSCUS MANICULATUS RUBIDUS (CARLOTTA STOCK)

		Black		White		Color		Red : Green	
	No.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
Wild[25]	38	85.56 ± .13	1.17	8.77 ± .06	.52	5.67 ± .11	1.01	3.39 ± .07	.61
Transplanted	41	86.23 ± .12	1.16	8.45 ± .07	.71	5.33 ± .09	.86	3.04 ± .04	.3[illegible]

With respect to the first three values (black, white and color), it is plain that the agreement between the two is fairly close. It is also plain that the slight differences between the earlier and later series are not due to a modification of the latter in the direction of the local race. The "transplanted" lot actually average slightly darker than the wild. On the other hand, the change in the red : green ratio is in the direction of *gambeli* and *sonoriensis*.

Whatever the significance of this last fact may be (if it has any biological significance), the condition of these pelages as a whole surely does not justify the conclusion that the introduced race has been modified by local climatic conditions.

Table VII renders possible a comparison between the wild and transplanted series with respect to the various measured characters other than coat-color. Owing to the considerably greater average size of the latter animals, it is particularly important, in the present case, that certain of these mean values should be reduced to the same standard body length (p. 491). Table VIII gives these corrected values for characters which require such correction.

In the case of this race of mice, it is plain that the linear measurements, with the exception of that for tail length, are significantly greater in the "transplanted" series. The mean tail length is slightly though not sig-

[25] See footnote under Table II.

TABLE VII

VARIOUS MEASURED CHARACTERS OF P. M. RUBIDUS (ACTUAL MEANS)

	No.[26]	Body length		Tail length		Foot length		Ear length		Tail stripe (%)		Foot pigmentation	
		Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
Wild	116	90.07 ± .31	4.89	93.58 ± .39	5.97	♂ 21.38 ± .06 ♀ 21.17 ± .07	.74 .78	17.27 ± .05	.88	41.53 ± .34	5.17	2.22 ± .10	.87
Transplanted	62	97.53 ± .36	4.27	97.86 ± .54	5.32	♂ 22.27 ± .08 ♀ 21.94 ± .07	.61 .54	18.13 ± .06	.66	48.21 ± .52	5.64	2.29 ± .07	.81

[26] See footnote under Table III. The figure for foot pigmentation, in the wild series, is based upon the same specimens (38) which were skinned. A considerably higher mean value is obtained when the entire lot, containing many immature individuals, are included. The feet are more heavily pigmented in young animals.

TABLE VIII

CORRECTED VALUES FOR TAIL, FOOT AND EAR IN P. M. RUBIDUS

	Tail	Foot (♂)	Foot (♀)	Ear
Wild	93.72 ± .39	21.52 ± .06	21.08 ± .07	17.23 ± .05
Transplanted	92.67 ± .54	21.89 ± .08	21.74 ± .07	17.76 ± .06

nificantly less in the latter series, while the depth of foot pigmentation is approximately equal in the two.[27]

When it is remembered that the Carlotta race considerably exceeds the La Jolla race in the length of the tail and foot, the width of the tail stripe and the depth of the foot pigmentation, it becomes evident that four or more generations of life at La Jolla have not modified the former race preponderantly in the direction of the latter. The single significant change in this direction (ear length) does not outweigh the greater changes in the opposite direction, particularly since ear length is the least characteristic of these racial differences.

In comparing the "wild" with the "transplanted" series of each race, certain statistically significant differences have been discovered, both in the case of *sonoriensis* and *rubidus*. It has been found, however, that these differences have not been preponderantly in the direction of agreement with the local race (*gambeli*), but rather in the contrary direction. Since, in respect to most of the characters here considered, *gambeli* is intermediate between the other two races, an inevitable consequence of these changes has been some degree of divergence between *sonoriensis* and *rubidus*.

A comparison of Tables II, III and IV with Tables VI, VII and VIII is instructive in this connection. It will be seen that in respect to all the characters under consideration, with a single exception, the transplanted series of *sonoriensis* and *rubidus* differ more widely from one another than do the wild series.[28] In the single case of the red: green ratio, the difference appears to be significantly less in the transplanted series.

It is well to explain at this point that I do not believe that the two introduced races of mice have actually become more unlike as a result of life at La Jolla. At least two explanations of this apparent divergence suggest themselves. In the first place, it is partly accounted

[27] See footnote under Table VII.

[28] All these characters showed a considerable initial difference, with the exception of ear length.

for by the greater modification (shortened appendages, etc.) which the *sonoriensis* stock has undergone as a result of captivity. On the other hand, the superior condition of the *rubidus* stock, at the close of the experiment (superior in some respects to that of the parent animals)[29] may have been the result of the survival of the sturdier strains in the course of four years' competition in the open pens.

As has already been pointed out, subspecific differences such as have been dealt with in the present paper have been attributed by some naturalists to local differences of environment, acting during the individual lifetime, or at most during a small number of generations. It is true that the environment in which these mice have been reared during the course of these experiments does not agree with the normal habitat of *P. m. gambeli* in the vicinity of La Jolla. Nor is the food by any means the same as that upon which they would subsist in nature, either here or elsewhere. These facts, however, are quite irrelevant to the present discussion. If any such prompt response to physical conditions actually occurred, we might reasonably look for a convergence of characters after the transfer of two geographic races to a common environment, even though that environment were a highly artificial one. That no such convergence has been detected during the period covered by these experiments is evidence of a greater degree of stability on the part of these characters than some writers have been disposed to admit.

The experiments have not, of course, established the probability that no such convergence would occur in the course of a vastly greater period of time, or under the influence of more extreme changes of environment. But they do create the presumption that mammalian subspecies in general,[30] and perhaps also of those birds,

[29] Not only the mean size, but the maximum size was considerably increased for both sexes.

[30] We have confirmatory evidence for one other species of *Peromyscus*, *P. eremicus*, the desert race of which has retained its original coat-color after several generations at La Jolla (Huestis, *Proc. Nat. Acad. Sci.*, October, 1923; Sumner and Huestis, *Biological Bulletin*, in press).

would show an equal degree of stability if their habitats were interchanged.

Artificially Modified Atmosphere

After it seemed probable that a simple transfer of these mice from one climatic region to another would fail to produce any appreciable modifications in their color, the possibility remained that positive results might follow the application of more extreme changes in the environment. Various distributional data, together with a meager amount of experimental evidence,[31] pointed to atmospheric humidity as a factor of probable influence in relation to the coat-color of mammals and birds.

Accordingly, a "desert room" was installed, with the object of reducing the relative humidity of the atmosphere to the lowest point practicable. This result was effected in two ways: (1) by warming the air of the experimental room, thus insuring a reduction in relative though not in absolute humidity; and (2) by circulating the air by means of an electric fan over a series of trays containing anhydrous calcium chloride, thus extracting water vapor from the atmosphere.[32]

The mean air temperature of the experimental room was 25.3° C., the mean relative humidity being 33.2 per cent.[33] In the control room, the mean temperature was 16.3°, the mean relative humidity being 73.8. The differ-

[31] Beebe, *Zoologica*, Vol. I, No. 1, September 25, 1907 (published by N. Y. Zool. Soc.); Bonhote, "Vigour and Heredity," London, 1915 (p. 86); Sundstroem, *Amer. Journ. Physiol.*, May, 1922 (p. 425–433). In each of these cases, animals were exposed to *high* humidity and an *increase* of pigmentation was believed to have resulted. Hollister's interesting observations upon cage-reared lions (Proc. U. S. Nat. Mus., vol. 53, June 1, 1917) may likewise be mentioned in this connection, though it is not certain that the darker color of these animals was due to the climatic changes involved.

[32] Quicklime (CaO) was employed as a dehydrating agent during the first half of the experiment.

[33] These figures are based upon thermograph and hygrograph records which are probably not very accurate. Only the records for the second year of the experiment have been here employed. They are compared with the La Jolla ("murarium") figures given in Table I, which are based upon two earlier years. This procedure seems sufficiently accurate for present purposes.

ence in relative humidity was thus very great, and the effects of this difference were obvious in the shrinking and "checking" of the wooden cages and the dryness of the hay which was provided as nesting material.

On the other hand, the reduction in *absolute* humidity was not as great as would have been desirable, owing to the limited capacity of the dehydrating agents for absorbing water vapor. The daily rations contained considerable moist food, and the excretions of the animals were doubtless by no means negligible in contributing to the water content of the air. Thus the latter averaged about 7.6 grams per cubic meter, a figure three fourths as great as that for the outside air (10.2 grams). The corresponding figure for Victorville is 5.9 grams per cubic meter, representing a considerably lower absolute humidity than that of my dry-room. As compared with Victorville, however, the *relative* humidity of the latter was much lower, averaging 33.2 per cent., instead of 49.8. These figures, representing the drying capacity of the atmosphere, are doubtless more important physiologically than the absolute amounts of water present.

This necessity for maintaining a high temperature in order to lower the relative humidity of the air was unfortunate for the purposes of the experiment. The conditions were frequently inimical to the health of the animals, a circumstance which was reflected in the greatly reduced fertility of otherwise prolific strains. On several occasions the temperature passed the danger point, and considerable numbers of the mice were killed by the heat.

Owing to the somewhat conflicting evidence from these experiments, the results will be presented rather briefly. It is my hope that a really adequate dry-room, together with another in which the humidity may be maintained near the saturation point, will be available for further investigations along these lines.

Peromyscus maniculatus dubius: Mice of this subspecies[34] were used most extensively, owing to their rela-

[34] *P. m. dubius* is a native of the Coronado Islands and of certain other islands near the coast of Lower California.

tively high fertility and normal development under conditions of captivity, as well as their more limited variability in respect to coat-color. It was early found that specimens transferred to the dry room when well grown underwent no appreciable changes in coat-color. Thenceforth, considerable numbers were reared from birth in the experimental room, some of these representing a second generation born in the latter.

In the first (juvenile) pelage it was noted that these dry-room mice tended to be appreciably paler than control animals of the same age. Numerous comparisons were made between broods belonging to the two contrasted lots, the difference being nearly always, though not invariably, in the same direction.[35] None of these juvenile skins were prepared, however, since it seemed more important to rear the animals to maturity, and, in general, the comparisons here referred to do not rest upon a perfectly secure statistical basis.

In the second (post-juvenile) pelage this initial difference almost invariably disappeared. With further molts, there was an undoubted reversal of the original relations, so that the dry-room mice, at the time of maturity, actually averaged somewhat darker than the control lot. They were likewise less highly colored, being of a more nearly neutral gray.

Despite a heavy mortality from heat, about a month earlier, 46 specimens of *P. m. dubius,* among those reared in the dry-room, were living when the experiment was discontinued in October, 1923. To the number stated, all of which were skinned at this time, should be added 8 which had been skinned earlier. The mice here considered ranged from 4 to 13 months old, some of them thus being far from mature. In the control series are 77 skins, ranging from 3 to 18 months in age.

For the purposes of examination, these two sets of skins were arranged in parallel series upon a table, each

[35] It is worth noting that some of the conspicuous exceptions belonged to the second generation born in the dry room.

of these being graded, according to age. A careful comparison of the two contrasted series fully confirmed the impressions which had been derived from the living animals.[36] When animals of the same age were compared, the dry-room series averaged slightly but unmistakably darker, as well as less highly colored, than the controls. This difference was least marked among the younger animals, and most marked among the older ones.

Since, in the normal course of development, the pelage tends to become paler and more richly colored, with the attainment of full maturity, the condition of the dry-room individuals suggested a simple retardation of development. Indeed, in many cases it was observed that the molt of dry-room animals was less far advanced than that of animals of the same age living under more normal conditions. A careful inspection shows, however, that the difference between these two sets of skins is not due to any mere difference of pelage phase. Not only are dry-room animals darker than control animals of the same age, but the former are darker at 13 months than the latter at 7 months.

Gambeli, heterozygous for albinism: The possibility occurred to us that an agency might affect the pigment formation of an animal having the "color" factor in a simplex condition, even though it might be incapable of producing this effect in an animal which was homozygous for the factor in question. For the purpose of such a test, albino *gambeli* were mated with wild-type mice of the same subspecies. The number of parent animals here employed was very small, so that no evidence for an experimental change of color could have been regarded as decisive, even if any differences of this sort had manifested themselves in the two sets of offspring under comparison.

Fifteen skins are available, derived from specimens which were either born in the dry-room or transferred

[36] It was not thought worth while to resort to careful analysis by means of the tint photometer.

there early in life. These have been compared with 20 controls. In each series, most of the specimens range from over 7 months to about 10 months in age, two being considerably older than this. In the present case, as in the previous one, the two series were laid out for comparison, each being graded according to age.

One's first impression, on viewing these two lots of skins, would probably be that the dry-room lot was paler. But this difference is due to the presence in the dry-room series of three exceptionally pale specimens. These three specimens can not, unfortunately, be compared with control ones of the same parentage, since there are none of these available.

More instructive is the comparison of members of "split" broods, *i.e.*, ones which were divided in early life, half of the individuals being placed in the dry-room and half used as controls. Skins are available for five such broods, aggregating 17 mice. While considerable individual variation in color is manifest (due perhaps to genetic segregation), there is no preponderant tendency for one series to be darker than the other.

Rubidus and gambeli (wild type): Small numbers of mice belonging to these two races were reared in the dry-room and compared during life with individuals reared in the control room. No differences were detected which could reasonably be attributed to environmental conditions.

Summary

(I) Mice belonging to the subspecies *Peromyscus maniculatus sonoriensis*, from the Mojave Desert, were reared for more than eight years at La Jolla, the resulting stock representing a minimum of seven and a maximum of twelve or more generations. During this period, they did not, in respect to any single measured character, undergo a modification in the direction of the La Jolla subspecies, *gambeli*. On the contrary, the mice of the later generations were in some regards less like *gambeli* than were their ancestors trapped in the desert.

(II) Mice belonging to the subspecies *P. m. rubidus,* from the northwest coast of California, were reared at La Jolla for six years, the resulting stock (with a very few exceptions) representing a minimum of four and a maximum of six or seven generations. Here again, the slight differences between the ancestral stock and its descendants were not such as to indicate a modification in the direction of the local race. As in the case of *sonoriensis,* the mice of the later generations of *rubidus* were, on the whole, less like *gambeli* than were their wild ancestors from Humboldt County.

(III) Comparing the two introduced strains, *sonoriensis* and *rubidus,* there was no tendency towards convergence, under the influence of a common environment. To judge from the samples at our disposal, there was actually a slight divergence in respect to all but one of characters which were measured.

(IV) The nature of these slight differences between the transplanted and ancestral series of a given race renders it highly improbable that they have been due to changed climatic conditions. To some extent, they are known to be the results of captivity, irrespective of climate.

(V) Subjection of deer-mice of several strains to an atmosphere of high temperature and very low relative humidity gave conflicting results. In *P. maniculatus dubius* the dry-room animals were, on the whole, noticeably paler than the control, while in the gray juvenile pelage. This difference was not invariable, however, and the numbers were not sufficient to furnish decisive evidence of such a change. In any case, this initial difference in shade disappeared with the assumption of the second pelage, while the difference was actually reversed in later pelages, the dry-room animals now being somewhat darker. Such an effect, of course, was quite unexpected, in view of the prevailingly pale coloration of desert mammals. But, in judging these results, the almost pathological character of the dry-room animals must be taken into account. In mice of certain other races, on

the contrary, no differences were noted between the experimental and control series, either in the juvenile or later pelages.

(VI) On the whole, one can not fail to be impressed by the comparative stability of these various races of mice under very marked alterations in the physical environment. As regards color characters, such almost wholly negative results are not in agreement with those of certain other experimenters who have reported pronounced color changes in animals, following considerable changes in atmospheric humidity. Nor do the present results afford any support for the view held by certain zoologists that the differences between geographic races or subspecies are purely "somatic" and therefore nonhereditary. Regarding the more difficult question whether climatic influences may not have a cumulative effect in the course of sufficiently great periods of time, our views must at present be decided by considerations of a taxonomic and distributional nature rather than by any available experimental evidence.

THE SEX CHROMOSOMES OF MAN[1]

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RECENT studies in human spermatogenesis have cleared up the doubt which has so long existed over the number of chromosomes possessed by mankind. The somatic or diploid number for the female is 48, and this must be taken as the basic number for the race since the female is homozygous for sex. There is not complete agreement as yet about the full diploid number of the male, both 47 and 48 chromosomes having been reported.[2] On the other hand, a haploid number of 24 chromosomes has been found uniformly in males when fresh properly preserved material was used for study, and includes germinal tissue (testes) from Europeans (d'Winiwarter, '12), American Whites and Negroes (Painter, '23) and Japanese (Oguma and Kihara, '23).

In view of the small size of the human germ cell and the complexity of its chromosome make-up, it is really surprising that observers, using very diverse methods of preservation, should agree so closely in spermatogonial counts. If spermatogonial counts were the only avail-

[1] Contribution No. 182 from the Zoological Laboratory, University of Texas, Austin, Texas. In carrying out these studies on mammalian chromosomes the writer has been aided by a grant from the Committee for Research on Sex Problems, of the National Research Council.

[2] D'Winiwarter and Oguma and Kihara have reported 47 chromosomes. The writer is the only investigator who has published a paper in which 48 was shown to be the diploid number. However, Evans is reported by Babcock and Clausen to have counted 48 chromosomes (''Genetics in Relation to Agriculture,'' ed. 1918, page 538). And Conklin reports that Guyer has written him that he has also found 48 in recent studies (''Heredity and Environment,'' 4th ed. 1922, page 166). As yet, so far as I know, neither Evans nor Guyer have published on this subject, but Professor Evans very kindly has shown me drawings of spermatogonia in which there were 48 chromosomes, and Professor Guyer has indicated to the writer his intention of publishing on this subject in the near future.

able evidence for the somatic number, it is doubtful whether or not we could ever be certain which reported number was correct, 47 or 48. In taking issue with d'Winiwarter's count of 47, and placing the full diploid number at 48, the writer was influenced, not so much by spermatogonial counts, as by the conditions observed in the first maturation division. Here I found the same haploid number as d'Winiwarter, but instead of a single X chromosome, which d'Winiwarter had inferred was present (but not observed), I found an X–Y sex chromosome complex. If the sex chromosome is paired in the male, as I have reported it, then the diploid number must be 48 (46 autosomes + X + Y). If, on the other hand, a single X chromosome is present, as originally reported by d'Winiwarter, and very recently maintained by Oguma and Kihara, then the diploid number is 47 (46 autosomes + X). It will thus be seen that the real point at issue is the question of the type of sex chromosome carried by the male sex. In either event, of course, the full diploid number for the homozygous sex (female) would be 48 (46 autosomes + 2X).

In view of the facts stated above, the central point of interest in human spermatogenesis now shifts from the question of chromosome numbers to that of the type of sex chromosome carried by the male sex. As long as there is doubt upon this second question, the matter of the exact somatic number must remain in an uncertain status.

The general matter of sex chromosomes in mammals has engaged the attention of the writer for some five years, and during this period a number of different mammalian forms have been investigated, in order, first, to show that sex chromosomes do really exist in this group (a fact for which we had previously no very conclusive evidence), and second, to gain as much information as possible on the form and behavior of these elements during all phases of spermatogenesis. Since sending my human study to press, over three years ago, several other investigations have been completed (either in press or

have just appeared) which have proved illuminating for the human condition. Study IV is especially interesting because it deals with the sex chromosomes of monkeys, and I have been able to give crucial evidence for sex chromosomes of the X–Y type in these lower primates. The form and behavior of these elements are essentially the same as in man. New human material has also been studied, in which I have found the same conditions reported in my earlier human work. Finally, direct genetic evidence and other cytological work have appeared which point indubitably to the conclusion that man (the male) carries a Y chromosome.

The several investigations referred to above had been in press some time before the recent paper of Oguma and Kihara appeared. In view of the fact that the conclusions of these investigators, as regards the sex chromosome, run counter to my own recorded observations for man and closely related animals, it has seemed wise to discuss in a general paper the question of sex chromosomes in man. Here it is proposed to review the evidence for sex chromosomes in the work previously done by d'Winiwarter, Oguma and Kihara, and myself, to indicate the nature of the results of my second human study, just completed, and to bring together and briefly summarize such other cytological or genetic evidence as may be pertinent to the question before us.

At the outset, of course, one must consider what is crucial evidence for the presence of sex chromosomes in any animal. In a study about to appear (Study IV) I have discussed this matter at considerable length, account being taken of variations from the usual sex chromosome conditions and of those anomalies in ordinary chromosome form and behavior which may, under certain conditions, simulate sex chromosomes. We may, therefore, omit the details and exceptions and state that there are, in general, five cardinal points which should be obtained in order to be sure that a given chromosome (or complex) is really the element which determines sex. Assuming

that the male is the heterozygous sex, these are as follows: (a) Spermatogonial counts and a study of the morphology of the individual elements usually indicate the type of sex chromosome which will be found in maturation.[3] That this point may not necessarily hold in lower mammals, at least, has been demonstrated recently by Agar ('23). In Macropus, the sex chromosomes sometimes fuse with autosomes, so that the spermatogonial number in the same individual appears to vary from 10 to 12 (the latter being the full diploid number). Under such conditions the observed diploid number would not be a reliable index of the type of sex chromosome. (b) The haploid count is needed as a check on the diploid number and when it is just half of the latter (for example diploid 48, haploid 24) indicates an X–Y sex chromosome. When the haploid is more than half the diploid, an X chromosome is indicated (47, 24). (c) The form and behavior of the sex chromosomes during maturation is the most direct and important evidence which one can obtain for sex chromosomes. If an unpaired X is present, it will be observed passing undivided to one pole in one of the maturation divisions. (In mammals the first is the reductional division for the sex chromosomes). Similarly, the X–Y elements, if such are present, may usually be identified as they segregate to opposite poles of the cell. If there has been an earlier association of the sex chromosomes with autosomes, it does not hold for this period. (d) Second maturation counts are needed to verify observation on the distribution of sex elements in the first division. (e) Finally, one must know the character of the female chromosome complex, in order to determine which is the X or female-producing chromosome.

Previous Work

D'Winiwarter counted 47 chromosomes in spermatogonia and 24 in the first maturation division. Secondary

[3] Thus an odd number, 47 for example, is suggestive of the X–O condition, or an even number (48) indicative of the X–Y type of sex chromosome. The form or morphology of the individual chromosomes should be studied, and homologous elements mated up (paired). When this last is done, chromosomes without mates of like size or shape can be identified.

spermatocytes showed either 23 or 24 chromosomes; hence he inferred that an unpaired X had passed undivided to one pole in the first maturation division. He did not identify this X chromosome. He found 48 chromosomes in the female.

Painter observed a diploid number of 48, and on pairing these up found that two were without mates of like size or shape. The haploid number was given as 24. In the first maturation division all elements appeared as tetrads except one, which was made up of two very unequal parts. These parts corresponded approximately in size to the unpaired chromosomes in spermatogonia, and were interpreted as X–Y sex chromosomes. The X and Y components were shown to segregate to opposite poles of the first maturation spindle. Second maturation counts were not made and the female condition was not investigated because of a lack of material.

Oguma and Kihara report a diploid number of 47. These were lined up and found to be paired except for the largest chromosome which they identified as the X. Twenty-four chromosomes were observed in the first maturation division, the largest element being again identified as the X. Its subsequent form and behavior was not followed.

At the time d'Winiwarter did his splendid work, the central point of interest was the question of the number of chromosomes characteristic of man, and questions of chromosome morphology and sex chromosomes were of secondary importance. Consequently, we find that the evidence which d'Winiwarter has given for sex chromosomes is what may be inferred from the numbers he observed in spermatogonia and in secondary spermatocytes. He never identified any particular chromosome as the X, nor did he observe it passing in an undivided state to one pole of the cell in maturation. His work may be harmonized with my own by assuming that he overlooked the very small Y in the second maturation division (see page 311 of Study II).

In my own work, when I found that d'Winiwarter's diploid count was approximately correct, I directed my attention primarily to the questions of chromosome morphology and behavior, especially during maturation, and the evidence which I have presented for the X–Y sex chromosome is based on direct observation of these elements as they segregated to opposite poles of the cell in the first maturation division. I lacked, however, the confirmation of second spermatocyte counts, and, of course, since I had not studied the female chromosomes, I could only infer (from conditions in the opossum) that the larger sex component was the X chromosome. This gap has been filled in part by my study of the lower primates, which will be referred to in detail later on.

The evidence for a sex chromosome of the X–O type which has been given by Oguma and Kihara rests mainly on spermatogonial evidence, the odd number (47) and the reported unpaired nature of the largest chromosome suggesting a single X chromosome in the male. Their evidence from the first maturation division can not be given much weight, because they did not follow the chromosomes beyond the metaphase, and did not show that any one of the 24 elements behaved as an X chromosome, *i.e.*, passed undivided to one pole. Furthermore, the chromosome which they have labeled "X" has the same sort of split which is shown in a number of other chromosomes in the same spindle. It is not clear why they call one element a single (unpaired) chromosome while the others are regarded as bivalents (tetrads). My own observations differ from these Japanese investigators in a number of points which will be taken up in the following section of this paper.

It is noteworthy that none of the investigators who have championed the X–O condition in man has observed the X chromosome passing undivided to one pole. The primary evidence for a sex chromosome of this type is found in the reported odd number of the spermatogonial chromosomes. On the other hand, I have been led to the X–Y interpretation because I observed in the first matu-

ration division the actual segregation of unequal sized components to opposite poles of the cell. The phenomenon was very closely similar to the condition observed in the opossum and was given the same interpretation.

NEW OBSERVATIONS

At the time the paper of Oguma and Kihara appeared I was engaged in a study of new human material (negro), so that I at once carefully checked up the points on which they disagree with me. The excellent illustrations of these Japanese cytologists have enabled me to identify their X chromosome and to follow it through the crucial period of maturation. In the following section of this paper, I shall briefly review the facts which bear upon the points at issue between Oguma and Kihara and myself.

Spermatogonia: The observations of Oguma and Kihara and myself for this period differ in three respects; the total number of chromosomes (47 *versus* 48), the condition (unpaired or paired) of the largest spermatogonial chromosome, and the matter (absence or presence) of a very small Y chromosome. In the foregoing pages I have presented cogent reasons why we can not hope to settle this first point of difference by spermatogonial counts. There are always points in the chromosome complex where one has to interpret the structures observed. In all the figures which Oguma and Kihara have given, except figure 3, one may with reason count 48 or even 49 chromosomes. Furthermore, and this applies to the third point of difference also, it is very possible that one or both of the sex chromosomes have a tendency to associate with some autosome during spermatogonial division, as in marsupials. This tendency may be more pronounced in some human races than in others. I have observed and figured spermatogonial cells in which I could find only 47 chromosomes, the missing element being the Y. I have interpreted such cells as cases where the Y was hidden by some overlying element, but it may well have been a case of association.

The second point of difference has reference to the largest chromosome. I have shown that it is paired, and a study of d'Winiwarter figures 15 and 16 seems to indicate the same condition as I pointed out in Study II. Oguma and Kihara maintain that it is unpaired, and have interpreted it as the X chromosome. Their figures show this element, however, as being very slightly if at all larger than several other chromosomes in the cell, and if we keep in mind the error due to foreshortening, it would appear that their identification of the X rests on very insecure ground.

Oguma and Kihara dismiss the matter of the Y chromosome with the observation that they found the smallest chromosome paired. An examination of my own figures (Figs. 31 to 41, for example) will show that I observed in addition to the Y a pair of very small chromosomes which approach the Y in size.

FIGS. 1 and 2. Spermatogonial chromosomes of negro. Forty-eight elements in each cell. The largest pair of chromosomes labeled 'a'. 'Y' is the small "male producing" chromosome.

Figures 1 and 2 show the typical appearance of spermatogonial chromosomes in my new negro material. Forty-eight chromosomes will be found in both of these cells, and the two largest chromosomes are labeled a. The number of apparent chromosomes does not always total 48; in some cases I have found 47 or even 46 elements, while in others, there were 49 elements. In no cell have I found that one chromosome was noticeably larger than the rest of the elements, though, of course, the form of the two largest chromosomes was not always the same. The smallest chromosome which I would interpret as the Y is so labeled in the figures.

Growth Period: In insects, as is well known, the sex chromosomes retain the condensed form during the so-called growth period of primary spermatocytes, and appear as densely staining masses which we shall call chromatin-nucleoli. Similar structures have been observed in mammalian spermatogenesis, and since Oguma and Kihara use this evidence to support their claim for a large X chromosome, we must consider the nucleolar history in some detail.

Although it has been quite generally assumed that the chromatin-nucleolus in mammals was made up of the sex chromosome material, definite proof of this has only recently been forthcoming. In Study III, the writer has given a very detailed account of the behavior and fate of the chromatin-nucleolus of the opossum, in which it was shown that it is really made up of the X and Y sex chromosome material. Its behavior, however, is different from similar structures in invertebrates in a number of minor particulars, notably in this, that the X and Y components unite in the early pachytene stage into a single mass which has a very labile form during late pachytene and diplotene stages. At no time during this relatively long period, when the chromatin-nucleolus is large and is the most conspicuous element in the cell, do we gain the least hint either of the form of the sex elements or of their final size. In Fig. 3 A to G, I give again, with a descriptive legend, the history of the chromatin-nucleolus of the opossum, because, as I will show below, the chromatin-nucleolus of man behaves in just the same way.

In man (Fig. 4) the chromatin-nucleolus arises from an aggregation of chromatin knots which lie on the polar side of the nucleus. These unite into a large more or less oblong structure which presents many different forms during the late pachytene and diplotene stages (Figs. 5 and 6). If one were to judge the size of the sex chromosome by the size of the chromatin-nucleolus during this period, as Oguma and Kihara have done, he would conclude with them that it was a very large structure. As

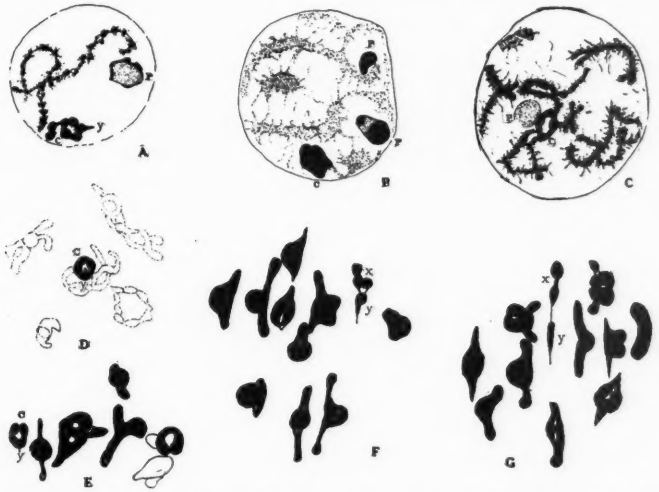
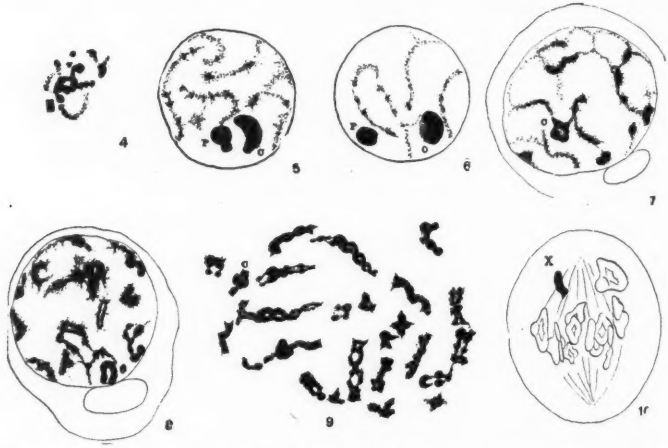


FIG. 3A to G. Showing the history of the chromatin-nucleolus 'c' in the opossum. A shows the formation of the nucleolus from knots of chromatin, the 'Y' element being labeled. B, late pachytene stage showing large size of chromatin-nucleolus 'c' and two plasmosomes 'p'. C and D are diakinesis stages; note reduction in size of chromatin-nucleolus. E to G show the way in which the chromatin-nucleolus forms the X and Y sex chromosomes.

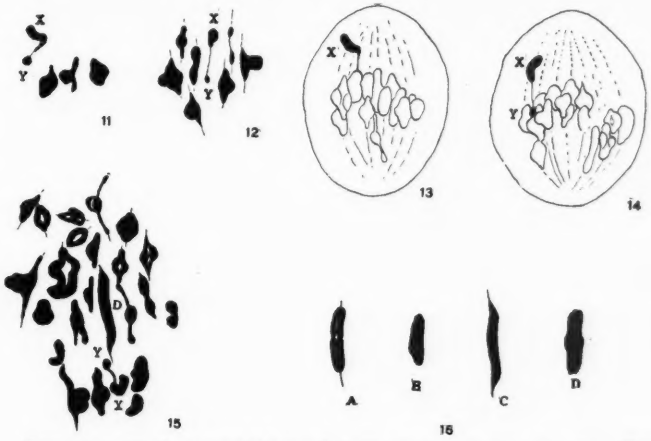
division approaches, however, the size of the chromatin-nucleolus decreases, probably by condensation and, perhaps, the loss of achromatic material, until by the early prophase it is a small structure (Fig. 7, "confused stage" and Fig. 8, early diakinesis). Note especially that in figure 8 the chromatin-nucleolus consists of a curved rod and a rounded element. Figure 9 is a late diakinesis stage which follows quickly after the condition shown in figure 8. (The chromosomes in this case have been drawn out separately, in order to show their form.) There are 24 elements present in this cell (from one section) which is the full haploid number. The chromatin-nucleolus is probably the structure labeled c, though I can not be sure in this. On the other hand, the longer chromosomes all consist of pairs of threads which are twisted together, this being of course the normal behavior of autosomes



Showing the history of the chromatin-nucleolus in man (negro). Only a small portion of the spireme threads are drawn. FIG. 4. Early pachytene stage showing formation of chromatin-nucleolus 'c' from a number of chromatin-knots. FIGS. 5 and 6 are late pachytene and diplotene stages showing large size of chromatin-nucleolus. 'P' are plasmosomes which disappear before spindle formation. FIG. 7, 'confused stage,' note small size of the chromatin-nucleolus 'c'. FIGS. 8 and 9, early and late diakinesis. FIG. 10, side view of first maturation division showing the true X chromosome. In this case the Y chromosome is not shown.

(tetrads) at this time. That the chromatin-nucleolus should have formed any of these larger chromosomes is inconceivable. Figure 10 is a cell which lay adjacent to that from which figure 8 was taken. The chromosome which I have interpreted as the true X is seen passing undivided to one pole. The Y in this case is hidden by overlying tetrads, but the point of interest is this, that the size of this X is about the same as that of the larger component of the chromatin-nucleolus (Fig. 8) from which it is undoubtedly derived.

Oguma and Kihara have used the chromatin-nucleolus at about the stage of figures 5 or 6, as evidence for a large X chromosome. The later history of this structure —which is quite similar to what occurs in the opossum— demonstrates, however, that it contracts and that by the time of diakinesis it must be considered as being among



FIGS. 11 to 12 show morphology of X–Y sex chromosomes of man. FIGS. 13 and 14, side views of first maturation division. The Y chromosome is not observed in figure 13, but note the heavy strand running from the X. FIG. 15 a 'spindle dissection' showing the 24 haploid chromosomes of man. FIG. 16A to D shows typical form of chromosome D, which is probably the element which Oguma and Kihara have identified as the 'X' chromosome.

the smaller third of the chromosomes. Figure 9 brings out the additional fact that all the larger chromosomes are made up of pairs of twisted threads. This is the most convincing sort of proof that all the larger spermatogonial chromosomes are paired.

First Maturation Division: The detailed study which I have made on new material for this stage has confirmed my earlier work. There are 24 elements in the spindle, 23 of which have the usual form exhibited by mammalian tetrads. One element is made up of two components of very unequal size (Fig. 11) connected together by a heavy chromatin strand. This is the X–Y sex chromosome complex which I found in two other individuals. Under normal conditions, the X–Y complex, being among the smaller chromosomes, occupies a position near the middle of the spindle, and can not be identified in side view unless the elements have already begun to segregate to opposite poles of the cell, or the spindle has been cut

so as to expose them to view. Figures 11 and 12 are cases of the latter sort in which both the X and Y are seen still connected by a heavy thread. As a usual thing in general side views of spindles one only sees the X going early to one pole, but it is always observed to be connected to the Y, which is frequently hidden in the equatorial plate, by a heavy chromatic thread (Figs. 10, 13 and 14).

Figure 15 is a spindle dissection of one of those rare cases where all 24 elements could be made out in a side view of the spindle. The X and Y elements are easily identified. All the larger elements in the first maturation division are tetrads, as was to be expected from the conditions observed in figure 9. The element which Oguma and Kihara have identified as an X is, as I interpret their figures, chromosome D of my Study II. It may be observed in figure 15, and it is noteworthy that it is drawn out at both ends by spindle fiber attachments. In figure 16, A to D, I give other characteristic forms of this chromosome. It is usually observed as a blunt heavy rod, often with a distinct split, as Oguma and Kihara have described. In favorable cells, however, its tetrad nature is revealed, as in figure 16D.

Anaphase stages of the first maturation division (Fig. 17) bear out the conclusions stated above. Figure 17 is

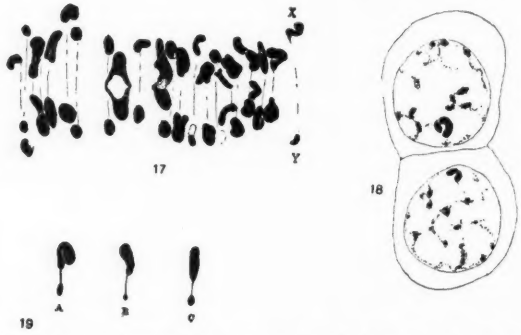


FIG. 17. Late anaphase of first maturation division showing the division of chromosome D. FIG. 18, resting stage of secondary spermatocyte. FIG. 19A to C shows the morphology of the X–Y sex chromosomes in primates. 19A, Brown Cebus; 19B, *Macacus rhesus,* and 19C, negro.

especially illuminating, as it shows chromosome D in the act of division. In this case the chromosomes have been separated to show their form. One may count 23 elements at each pole of the cell. I have been unable to find the two parts of chromosome W, which is the smallest tetrad in the human spermatocyte. At the top of this figure the X chromosome is observed, while its mate, the Y, is on the lower side. It is not always possible in late anaphase and telophase stages to count the full haploid number at each pole of the cell, but enough of these stages have been studied to show that, as in figure 17, all the larger chromosomes divide. If a very large X chromosome, such as described by Oguma and Kihara, had passed undivided to one pole of the cell, it could hardly be overlooked because of its size.

Second Maturation Division: So far the only evidence which I have to present for this period is that found in the rest stage of the interkinesis period just before the second maturation division. In figure 18 the two daughter cells of a first spermatocyte are shown. In one cell there is a large chromatin-nucleolus, presumably the X, while in the other daughter there is a much smaller Y.

Many secondary spermatocyte divisions have been studied, but in all cases so far, there has been marked irregularity in the time at which the individual chromosomes divide. This has precluded the possibility of decisive counts, such as are needed to prove that 24 chromosomes are always present in these cells.

Other Evidence

In addition to the evidence which is found in a study of human spermatogenesis, the conclusion that man possesses the X–Y type of sex chromosomes is further supported by the fact that such sex chromosomes are found in the lower primates and in a number of other mammals.

Sex Chromosomes of Monkeys: The lower primates, of course, have a more direct bearing upon the sex chromosome condition of man than any other form. The fact is, that I was led to a study of the chromosomes of monkeys

because it seemed probable that if the structures which I identified in man really had the fundamental function which I attributed to them, similar elements would be found in other primates. Preliminary reports covering both new world and old world forms have been published (Painter, 22b and 23b), and the completed work (Study IV) will probably have appeared by the time this article is printed.

Both the new world and old world monkeys show in their spermatogenesis the same sort of condition which I found in man. The spermatogonial number is even, and when the individual elements are paired up, two remain without mates of like size or shape. Among the tetrads, during the first maturation division, one element is found which is made up of two unequal parts. Figure 19A shows the form of this in a new world monkey (*Brown cebus*), 19B the condition in the *Macaccus rhesus,* and for ready comparison, figure 19C the condition in man. The similarity of the elements is obvious from figure 19, and their behavior in maturation is identical. In the case of the *Macaccus rhesus,* the somatic chromosome complexes of both male and female embryos were also studied. These confirmed the observations made in spermatogenesis. Forty-eight chromosomes (spermatogonial number) were found in both males and females. These two sexes differ in this respect, that while the male possesses two chromosomes which have no mates of like size or shape (a medium sized rod and a very small ball) the chromosomes of the female are all paired. In other words, the female carries two X chromosomes, while the male has an X and a Y. This work is of further interest as it shows that the larger of the two sex components is the female-producing or X chromosome, the smaller being the Y.

No more striking confirmation could be asked for than that given by the lower primates. Here the evidence for sex chromosomes of the X–Y type is complete, and the close similarity of both the form and behavior of the X and Y components, in man and in the monkeys, makes it

apparent that we are dealing with elements which must have a fundamental significance in all primates.

Sex Chromosomes in Marsupials: The opossum was the first mammalian form for which complete crucial evidence for sex chromosomes was given (Painter, '21), the X–Y type of sex chromosomes being demonstrated. Very recently Agar ('23) and Greenwood ('23) have given the results of their studies for five different Australian marsupials. In each of the species studied—and in most of these cases the female chromosome complex is also described—there is an X–Y sex chromosome complex. It may be pointed out further that, as in the case of the opossum, these sex elements were the smallest chromosomes in the cell. In figures 21 and 22 the X–Y sex chromosomes of Macropus are shown. (Taken from material kindly sent the writer by Professor W. E. Agar.)

FIGS. 20 and 21, showing X–Y sex chromosomes of *Macropus ualabatus.* FIG. 22 shows the X chromosome in the albino rat.

Sex Chromosomes of the Horse: In Study V, now in press, the writer has described, for the horse, an X–Y type of sex chromosome. The evidence was not as complete as for the forms cited above, but a typical X–Y chromosome was observed in the first maturation division quite similar in form and behavior to that found in the opossum and in the primates.

In all the cases so far cited, X–Y sex chromosomes have been found, and in each case the X has been a relatively small chromosome and the Y component an exceedingly minute element. It would not be surprising, therefore, if in some mammals we should find that the Y had been lost. The rat appears to be an animal in which this has occurred. Allen ('18)) in his spermatogenesis of the rat

shows that only the X is present. In this case, however, the X is not the largest chromosome, but is a medium sized structure. In figure 23, a drawing of the sex chromosome of the rat is given (this cell was found in a slide presented to the writer by Dr. Ezra Allen). The X chromosome of the rat has proportionally about the same size, as in the case of the primates.

Genetic Evidence: The presence of sex-linked characters in man, such as color blindness, haemaphilia, etc., have been known for a long time and have shown that the male sex carries one X chromosome, *i.e.,* is heterozygous for sex. It is only comparatively recently, however, that genetic evidence has been found which demonstrates that in certain vertebrates the Y chromosome may carry genes.[4] The best known cases of this sort are found in teleosts, and have been carefully worked out by Schmidt ('20), Aida ('21), Winge ('22) and others. The only other vertebrate for which a similar condition has been reported is man, and this case rests upon one family history involving four generations described by Schofield ('21).

A father having a certain character (webbed toes) transmitted it to all his sons and none of his daughters. The sons, in turn, transmitted this character to all their sons and none of their daughters. The daughters from the several generations involved never showed the character, nor did they transmit it to any of their offspring. If the case has been correctly reported, as there is every reason to believe, the only possible explanation for this case is that the defect was carried by the Y chromosome of the male, as pointed out by Castle ('22).

Other cases of webbed toe inheritance have been reported in which the distribution of the defect did not show a sex chromosome transmission. Wright ('22) has pointed out, however, that the same outward expression of a character may be due to two entirely different genes,

[4] In this respect the vertebrates differ from the invertebrates, because so far as we now know, the Y chromosome of invertebrates carries no genes.

and explains the different pedigrees which have been reported for webbed toes on this basis.

General Conclusions

In the foregoing pages a considerable volume of direct and indirect evidence has been presented which indicates that the sex chromosomes of man are of the X–Y type. It will scarcely be necessary to summarize this evidence here, but we must again emphasize the point that the cytological evidence is based upon direct observation on these elements during maturation. Those who have claimed that there was a single X chromosome in man have based their conclusion primarily upon chromosome counts in spermatogonia.[5] The evidence for a single X chromosome is thus largely inferred, since none of the writers advocating this interpretation have observed it passing undivided to one pole of the cell during maturation.

Since all recent investigators on human spermatogenesis agree with d'Winiwarter that the haploid number is 24 chromosomes, it follows that the true diploid number for the male is 48 chromosomes as I have reported it (46 autosomes + X + Y). When 47 chromosomes are observed in spermatogonia, it probably means either that one element has been overlooked or else that one of the sex chromosomes is temporarily associated with some autosome, as is the case in certain of the lower mammals.

LITERATURE CITED

Allen, Ezra.
1918. Studies in the cell division of the Albino Rat. III. Spermatogenesis. *Journ. Morph.*, Vol. 31.

Agar, W. E.
1923. The Male Meiotic Phase in two genera of Marsupials. *Q. J. M. S.*, Vol. 67.

Aida, T.
1921. Sex-linked Inheritance in Fresh-water Fish. *Genetics*, Vol. 6.

[5] In this paper I have not considered the earlier studies in human spermatogenesis. A full discussion of these works will be found in Study II beginning with page 310.

Castle, W. E.
1922. The Y-chromosome Type of Sex-linked Inheritance in Man. *Science*, Vol. 55.
Greenwood, A. W.
1923. Marsupial Spermatogenesis. *Q. J. M. S.*, Vol. 67.
Oguma and Kihara.
1923. Étude des chromosomes chez l'homme. *Arch. de Biol.*, Vol. 33.
Painter, T. S.
1922a. Studies in Mammalian Spermatogenesis. I. The Spermatogenesis of the Opossum. *Journ. Exp. Zool.*, Vol. 35.
1922b. The Sex Chromosomes of the Monkey. *Science*, Vol. 56, page 286.
1923a. II. The Spermatogenesis of Man. *Journ. Exp. Zool.*, Vol. 37.
1923b. Further Observations on the Sex Chromosome of Mammals. *Science*, Vol. 58, page 247.
1924a. III. The Fate of the Chromatin-nucleolus in the Opossum. *Journ. Exp. Zool.*, Vol. 39.
1924b. V. The Chromosomes of the Horse. *Ibid.*, Vol. 39.
1924c. IV. The Sex Chromosomes of Monkeys. *Ibid.*, Vol. 39.
Schmidt, J.
1920. C. R. Trav. Laboratoire Carlsberg, Vol. 14.
Schofield, R.
1921. Inheritance of Webbed Toes. *Journ. Heredity*, Vol. 12, page 400.
D'Winiwarter, H.
1912. Études sur la Spermatogenèse humaine. *Arch. de Biol.*, T. 30.
Winge, O.
1922. One-sided Masculine and Sex-linked Inheritance in Lebistes. *Journ. Genetics*, Vol. 12.
Wright, S.
1922. Another Pedigree of Webbed Toes. *Journ. Heredity*, Vol. 13.

GYNANDROMORPHS FROM X-RAYED MOTHERS

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IN the extensive work on Drosophila two kinds of individuals have been found which can not be classed as either male or female. Of one kind are the intersexes discovered and investigated by Bridges (1921). In an intersex the body as a whole shows a structure intermediate between that of the male and female. Of another kind are the gynandromorphs investigated in detail by Morgan and Bridges (1919). In a gynandromorph a part or parts of the body may be female in their structure and a part or parts male. The occurrence of both intersexes and gynandromorphs has been shown to be associated with a corresponding abnormal distribution of the chromosomes. Bridges (1921) has shown intersexes to be due to an abnormal proportion between the numbers of autosomal and X-chromosomes. Morgan and Bridges (1919) have collected a large body of evidence showing that the occurrence of gynandromorphs is due to nondisjunction or elimination of the X-chromosomes in some of the body cells during development.

The writer has already shown that X-ray treatment may induce abnormal distributions of the X-chromosomes at the time of maturation of the egg (Mavor, 1924). In the course of these experiments a number of gynandromorphs have occurred in the offspring of the X-rayed females and none in the controls. Although the number of gynandromorphs is small, it is believed their occurrence is evidence that X-ray treatment may induce the development of gynandromorphs.

To date in our X-ray experiments we have found four gynandromorphs among the F_1 of X-rayed females and none among the F_1 of control females. The first occur-

rence of gynandromorphs in our experiments has already been reported (1924). The two gynandromorphs occurred among the F_1 of X-rayed females in our first series of experiments. In this series of experiments wild type females were X-rayed for four minutes at 2.5 M.A. and 50 K.V. at a distance from the tungsten target of between 3.9 and 5.4 cm and mated to white-eyed males. According to our way of estimating dosage this dose is represented by 34.2–65.7 D. The total number of F_1 produced by the 22 control females was 7,340 red-eyed plus one exceptional white-eyed male. By the 19 fertile X-rayed females the number of F_1 produced was 2,883 red-eyed, 24 exceptional white-eyed males and 2 gynandromorphs. The two gynandromorphs were both of the bilateral type, one side being predominantly male and the other female. One of these, No. 1, is illustrated in

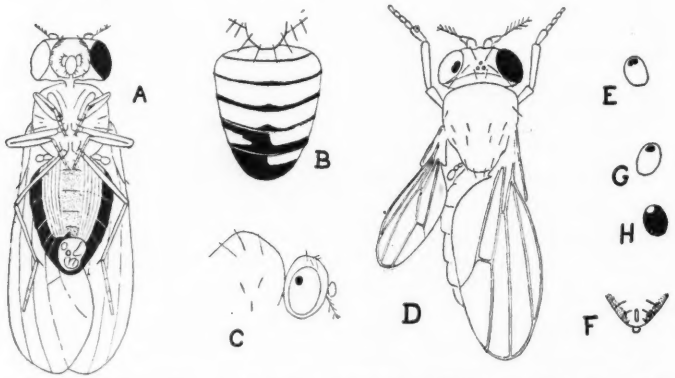

FIG. 1. Gynandromorphs from X-rayed mothers. A–C: Gynandromorph, No. 1, found in first series of experiments; A—ventral view; B—dorsal view of abdomen; C—right side of head. D–F: Gynandromorph, No. 5, found in a later experiment (VIth series); D—dorsal view; E—left eye; F—ventral view of posterior of abdomen. G, H: Gynandromorph, No. 6, found in the same experiment as No. 5; G—right eye; H—left eye.

Fig. 1, A, B and C. It arose as stated above from the mating of an X-rayed wild type mother by a white-eyed father. The right side was male, the left female. Presumably the maternal X-chromosome, the X-rayed

X-chromosome, became eliminated from some of the somatic cells, mostly those of the right side during early development. The second gynandromorph, No. 2, obtained in this series of experiments was essentially similar to the first.

Recently gynandromorphs have been again obtained among the F_1 of X-rayed females. In this case the females were X-rayed while in the pupa stage for ten hours with the current at 1 M.A. and 50 K.V. at a distance of 50 cm from the tungsten target. According to our method of recording dosage this is represented by 24D. The X-rayed females which were heterozygous for white and eosin eye-color, long and miniature wings, were mated to wild type males. The total number of F_1 produced in the 25 control matings was 9,627 regular males and females, four exceptional males and one exceptional female. In the case of the 107 females which were X-rayed the number of F_1 were 24,336 regular males and females, 118 exceptional males, 13 exceptional females and two gynandromorphs.

These two gynandromorphs were also of the bilateral type. One of them, No. 5, is illustrated in Fig. 1, D, E and F. Here the left side is predominantly male and the right female. Clearly the maternal chromosome carried white and miniature, being a crossover chromosome. The external genital organs were female (Fig. 1, F). The other gynandromorph which occurred in this experiment was predominantly male on the right side and female on the left. The right eye (Fig. 1, G) was white with a dorsal red spot. The left eye was red with a white spot corresponding in position and size with the red spot on the right eye (Fig. 1, H). A sex comb was present on the right fore leg and absent on the left. The wings were of approximately equal size. The external genital organs were male.

It is to be noticed that the X-chromosome which was eliminated in the male parts of these two gynandromorphs was a paternal chromosome which had not been exposed to X-rays. Hence if these gynandromorphs were due to X-rays the action must have been indirect, the

X-rays having produced in the egg a condition which subsequently led to the elimination of an unexposed X-chromosome.

A large number of experiments have been performed in which females were X-rayed and no gynandromorphs appeared among their offspring. The technique of the X-ray treatment has been changed from time to time in the course of the investigation so that statistical treatment of the combined results is rather unsatisfactory. However, the only statistical comparison which seems justifiable is one involving the F_1 of all the X-rayed and all the control females. If the data of all our X-ray experiments be added together it is found that 373 females were X-rayed and produced 68,186 F_1, of which four were gynandromorphs, while 237 control females (in each experiment the control females were sisters of the X-rayed) produced 65,128 F_1, of which none were gynandromorphs. A formula developed by Karl Pearson (1907) for the probable error of the difference is particularly applicable to a case such as this since it takes into account the smallness of one of the classes, in this case the gynandromorphs. This formula gives 2.99, or approximately 3, as the difference between the number of gynandromorphs produced by X-rayed females and the mean expected of them from the number produced by the controls, divided by the probable error of the mean. Expressed in terms of probability, this gives a probability of approximately 20 to 1 that the difference is due to the treatment.

It should be mentioned that two other gynandromorphs have occurred in our laboratory—one was the F_2 of a control female, and the other occurred in connection with a classroom experiment. Since the data involving the occurrence of these is of quite a different nature from that given above they have not been included in the statistical treatment. It is to be noted that the occurrence of the gynandromorphs among the F_1 of X-rayed females, approximately 1 in 17,000, is not as frequent as gynandromorphs have been observed to occur in nature. Morgan and Bridges (1919) record a case where they were

found as frequently as 1 in 1,325. The evidence for X-rays acting as an agent inducing the development of gynandromorphs must rest entirely on a comparison of the behavior of control and X-rayed females genetically identical and reared under identical conditions. The fact that gynandromorphs have occurred more frequently under other cultural conditions than they have been found among the F_1 of X-rayed females merely shows that X-rays are not as effective an inducing agent as certain other unknown factors which may be present in cultures.

The occurrence of gynandromorphs as the result of X-ray treatment is of interest from more than one point of view. If the indications of these experiments should be substantiated by the further finding of gynandromorphs among the F_1 of X-rayed females the following conclusions would seem justified: (1) X-rays, in inducing the elimination of the X-chromosome, and probably in inducing non-disjunction, do not act directly on the chromosomes but rather by producing a condition which subsequently leads to the observed effect on the chromosome; (2) the Morgan–Bridges theory of the occurrence of gynandromorphs as due to the elimination or non-disjunction of the X-chromosomes during development, already well substantiated, would receive additional support if it is proved that a physical agent which is known to induce non-disjunction of the X-chromosome also induces the formation of gynandromorphs.

LITERATURE CITED

Bridges, Calvin B.
1921. "Triploid intersexes in *Drosophila melanogaster*," *Science*, N. S., Vol. LIX, pp. 252–254.

Mavor, James W.
1924. "The production of non-disjunction by X-rays," *Journal of Experimental Zoology*, Vol. 39, pp. 381–432.

Morgan, Thomas H., and Bridges, Calvin B.
1919. "The origin of gynandromorphs," Carnegie Institution of Washington, Publication Number 278, pp. 1–122.

Pearson, Karl.
1907. "On the influence of past experience on future expectation," *Phil. Mag.*, 6th ser., Vol. 13, pp. 147–209.

COMMUNICATION BY SCENT IN THE HONEY-BEE—A THEORY[1]

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A NUMBER of minute unicellular glands open upon the intersegmental membrane between the seventh and eighth terga of the abdomen of the honey-bee. These glands are called the glands of Nassanoff, after their discoverer. A number of suggestions have been made as to their possible use, but it remained for Sladen[2] to establish the fact that they are scent-producing organs. Sladen found that these organs give off a strong odor even when the part of the back to which they are attached is removed from the abdomen. He also asserted that this odor is the same as that which is given off when a number of bees are shaken to the ground before a hive.

Under these circumstances, as well as at the time of natural swarming, bees were known to produce a peculiar sound, sometimes called the "joyful hum." Sladen observed that this sound is produced by those individuals which first find the entrance to the hive, later by those next to them, and finally by others farther to the rear, so that soon all are informed of the location of the entrance into which they then make their way. He also observed that the odor mentioned above is emitted at this time and accordingly he asserted that it is the odor and not the sound which is the real means of information. He held, furthermore, that the sound is incidental to the special movement of the wings produced for the purpose of blowing the odor away from the body. He argued that we

[1] The author's thanks are due to Dr. E. F. Phillips, of the Bureau of Entomology, and to Dr. S. O. Mast for assistance in the preparation of the manuscript.

[2] Sladen, F. W. L., "A scent-producing organ in the abdomen of the worker of *Apis mellifica*," *Ent. Mag.*, Lond., Vol. 38, pp. 208–211.

have no evidence of an acute sense of hearing in bees, but that it is a well-established fact that they do possess a delicate sense of smell. Subsequent investigations have confirmed Sladen's observations, and his assertion that odor in this case is the real means of communication also is accepted generally.

Recently v. Frisch[3] has ascertained that the use of this scent has a wider application. He asserts that when bees detect a new source of food they expose this gland and by fanning distribute the scent so that bees in the vicinity are attracted to the food. In this way sugar syrup or blossoms without odor, if discovered even by a few bees, are soon known to many gatherers. Park[4] records an observation of similar behavior for bees carrying water, although in this case he states that the bees attracted were within a radius of eight or ten inches.

During the summer of 1923 the observations of v. Frisch and Park were confirmed by experiments conducted at the Bee Culture Laboratory of the Bureau of Entomology. Not only was it shown that bees attract others in their vicinity by emitting an odor while feeding, but it appears that the odor is a most important factor in enabling the discoverer of supplies to lead others directly to them. In other words, it appears that the discoverer of such supplies produces a scented trail through the air, thus enabling other bees to follow it. So fantastic is such a theory that one hesitates to announce it, were it not that the facts observed are of such a nature as well-nigh to establish such a theory as a fact.

Before proceeding to a consideration of such behavior in honey-bees, it is well to consider some of the conditions which must obtain in order that one animal may follow another by scent. Among such conditions are the following: (1) The creature followed must possess a scent; (2)

[3] v. Frisch, Karl, "Über die 'Sprache' der Bienen," in Munchen, Med. Wochenschr., 1920, pp. 566–569. Also in book form, 1923, Gustav Fischer, Jena, pp. 1–186.

[4] Park, Wallace, "Communication among bees," in *American Bee Journal*, 1923, Vol. 63, p. 449.

this scent must be of such strength and character as to be perceptible to the individual which is following; (3) the scent must possess sufficient permanence to enable the pursuer to pick it up after the pursued has passed; (4) such a trail, although it may be broken, must possess a degree of continuity sufficient to enable the pursuer to cross such breaks. These points may now be considered with reference to the honey-bee.

That the honey-bee possesses a scent is generally accepted. Not only does it possess a body-scent, as do many other creatures, but it also possesses a special scent-producing organ, as previously noted. According to Sladen,[2] Shaftesbury[5] and v. Frisch[3] and the present writer's observations this gland produces scent of quantity and quality such as to be perceptible to man. This being true, we have the possibility of scent production in the bee of such quantities that considerable dilution may occur and yet sufficient strength be retained to be perceptible to another bee. It is well established that honey-bees have a far more acute olfactory sense than has man.

Regarding permanence of the scent trail, facts favor the present theory when contrasted with conditions under which other animals follow scent trails. In well-known cases of tracking by scent, the trail often remains for hours, as for example, in tracking by bloodhounds. In the case of the bee, however, permanence of trail need be a matter of minutes only, or even of seconds. Observations of the writer confirm the findings of other investigators who assert that bees which act upon the information received from the discoverer of a new source of supplies, do so promptly, that is, they usually leave the hive within a few seconds. Occasionally they defer the start for as much as two minutes (Park[6]). Such a delay, however, is about equal to the average time required by

[5] Shaftesbury, A. D., "Some habits of honeybees," Thirteenth Annual Report of the Md. State Beekeepers Assn., 1922, pp. 11–22.

[6] Park, Wallace, "The language of bees," in *American Bee Journal*, 1923, Vol. 63, p. 227.

a gatherer to deposit a load of nectar within the hive. Such a delay, accordingly, enables the associate gatherer to leave at approximately the same time as the discoverer. Whether the associate leaves promptly or whether it defers its departure until the discoverer leaves again, in either case it is able to pick up a fresh trail leading from or to the source of supplies, if such a trail exists.

If a trail possessing a permanence of from two to three minutes is all that is required to support the present theory, there remains to be considered the possibility of the trail having sufficient continuity to enable another bee to follow it. This point is supported by analogy, and for this purpose the observations of hunters may be cited. In hunting, game sometimes passes within clear view of the hunters so that its path may be seen. Not infrequently is it observed that the dogs run in a course parallel to that taken by the quarry and at a distance of a hundred or more yards to one side of it. So striking is this behavior that were it not explainable on a physical basis, one would be justified in the assumption that dogs are indifferent trailers. The apparent discrepancy is explained when it is realized that winds which are passing across the path of the quarry carry the scent to leeward and that the directing influence in this case is not actual footprints but is in reality a movable column of scented air connecting the starting point of the chase with the fleeing animal. Supporting evidence for the carriage of scent which is perceptible for great distances is also found in the fact that game is apprised by scent of the approach of hunters, while they are yet at distances of a half mile or more. Trails in the snow likewise have established the fact that certain carnivorous animals are attracted to their prey over equal distances. Such windborne scent is of such a nature as to enable the creature even to locate its prey.

In further consideration of a movable column of scented air it may be helpful to study the action of a similar column of visible gases. Such a column may be noted

along railroads on cool mornings, when the steam from passing locomotives lags behind in a long drawn-out column. This column, still maintaining its continuity, is frequently carried hundreds of yards by wind blowing across the course of the railroad. The visibility of this column is at length lost, but this is often due to the evaporation and precipitation of its particles rather than to the particles being scattered by the winds. A still better example, although a less common one, is that of sky-writing, which has been introduced recently. In this case the conditions are almost identical with those under which our hypothetical "tracer-bee" would have to work. In sky-writing an aeroplane is used, and from this smoke or visible gases are released; the machine being maneuvered at the same time so as to describe the letters or figures required. Such tracings persist to the extent that they may be carried by the winds for a mile or more. Not only do these tracings retain their visibility during this time, but the various parts of the individual letters retain their identity. This evidence shows that there is little tendency towards the breaking up of such columns of air. The effect of the winds is principally that of transportation of the column as a whole rather than a dissipation of its component parts. This being the case it is reasonable to suppose that a scent-ladened column, although invisible, has equal or greater persistence than the visible column described.

It has been shown that columns of smoke persist for periods of several minutes, the length of such persistence being dependent upon atmospheric conditions. It has been shown also that columns of scented air likewise are known to persist for considerable periods. It now remains to examine the evidence tending to show that honey-bees produce a column of scented air which serves to direct a fellow-worker to the location of newly discovered supplies.

As already pointed out, Sladen shows that a scent is emitted and that it serves to attract other bees to the en-

trance of the hive. Von Frisch and Park assert that this scent is also used to attract bees over limited areas to the location of supplies of honey, nectar, sweetened water and pure water. All these observations have been confirmed by the present writer on many occasions. It is also apparent that the bees use this scent gland at times when they are not actually in contact with the supply of food.

In one set of experiments a maze was introduced at the entrance to the bait-box. Through this maze the bees at first were reluctant to pass, but under these circumstances it was observed that more than the usual amount of "fanning" was exhibited by bees which had gained entrance to the box. Not infrequently a bee would remain fanning for 30 or 40 seconds at the inner end of the maze, and during this fanning the scent gland was continuously exposed. During and shortly after such fanning, newcomers were directed more readily to and through the maze. This fanning often became so violent that the claws of the bee were insufficient to anchor it to the floor of the box, in which case the bee fell forward, often making a complete somersault. In righting itself it almost invariably came up with its scent gland still exposed. Occasionally, a bee was seen poised on the wing within the box and with the scent gland exposed.

In another set of experiments not more than one bee was allowed in the box at one time. It was observed that some of the excluded bees flying about the bait-box had their scent glands exposed. Finally, it was observed that the scent glands of some of the arriving bees were exposed, while the bees were yet on the wing and before they had alighted on the platform on which the bait-box had been placed.

These observations are significant, since they show that the scent gland is actually operated while the bee is on the wing and especially that arriving bees sometimes have their scent glands exposed. It may then be inferred that the scent glands are in operation continuously from

the time the bees left the vicinity of their hive. The difficulty attendant upon a check-up on this inference is great; since the bees fly at considerable height and at a rate which makes observations difficult if not impossible. The point that this particular behavior may at times be seen clearly when they do slow up in preparation for landing is a point which can not be ignored.

The theory of a scented trail is strengthened by the apparent elimination of other theories which have been proposed, that is, the sight theory and the theory of a general search. The theory that the hive-mate follows the discoverer by sight has been practically abandoned, since it is established that the hive-mate does not accompany the discoverer, but that it usually leaves the hive either before or after the discoverer and also that it frequently arrives at the bait after the discoverer has left for the hive. The theory of a general search for discovered supplies is weakened by the frequently recorded observation that at first only a few bees respond to information of such supplies by going to the fields. This fact discredits the belief that such bees search indiscriminately in all directions until by chance the supplies are found. If such conditions actually were to obtain, these two or three bees must search thoroughly more than five hundred acres of surrounding territory to locate a practically scentless bait of sugar syrup placed at a distance of one half mile from the hive. Not only would the first hive-mate be required to locate such supplies by chance, but each additional bee would likewise be required to find the supplies by chance. This condition would exist until a line of flying bees were established which could be followed by sight.

During the experiments at Washington it was observed that when two locations are desired at which to feed or bait bees from a particular hive, it is best to catch one or more bees from in front of the hive and carry them to the location desired. In no case was it found practicable to establish baits at various points and then introduce

bees at one of them, hoping that in a general search bees from the same hive would locate the other baits. Such a proceeding is, moreover, wholly at variance with the well-established practices of bee-hunters when establishing cross-lines.

In conclusion it should be stated that exposed scent glands were not observed on bees during their flight between the hive and the location of newly discovered supplies, except that exposed scent glands were observed on bees coming from the hive when they were within a few feet of the food supplies and after they had slowed down in preparation for landing. The inference may be made, however, that the discoverer does emit an odor from its scent gland throughout its passage between the supplies and its hive. It may be inferred, further, that such an odor enables a hive-mate to follow such a discoverer readily, and that it is in this manner that additional bees are led to the supplies.

VARIATIONS IN THE PREMAXILLARY OF EURYCEA BISLINEATA[1]

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IN my study of skulls of large numbers of *Eurycea bislineata* in connection with a more extensive morphological problem, I have come across an occasional example of premaxillary which deviates from the usual fused type for this species (Figure 1, a and b) in that the two ascending processes are joined along their medial borders to a greater or lesser extent, thus completely inclosing a fontanelle. These examples (Figure 1, c, d and e) present in the more extreme cases an appearance quite different from the usual type and closely resembling that of *Pseudotriton ruber* (Figure 2, c) and *Desmognathus fusca.*

At first these cases seemed to me to have little significance, since from the widely separated position of the ascending processes in the typical larval stage there normally occurs, simultaneously with the structural changes leading to metamorphosis, a gradual approximation of these two processes so that they come to lie near the midline, and the united condition seemed to indicate only a slightly more extensive ossification in certain individual cases resulting in the fusion of the processes. When, however, younger individuals were found in which this same unusual type of premaxillary occurred, I was led to consider the possibility that this was a matter of deeper significance than a chance difference in the degree of ossification.

I have, therefore, examined recently all the individuals constituting two representative collections of larvae of *Eurycea bislineata* from a single locality, Bears' Den

[1] This paper is No. 117 of the Contributions from the Department of Zoology of Smith College.

Brook, on Mt. Toby, in Sunderland, Massachusetts. These collections, made in June and August, respectively, of 1916, comprised together a fairly complete series of 109 individuals ranging from recently hatched larvae of about 15 mm in length to metamorphic individuals probably from two to three years old and measuring from 50 to 60 mm. They had been preserved in formalin and subsequently stained *in toto* in alizarine for the study of bony structures, which are thus differentially stained. Each of these was examined by the simple process of stripping

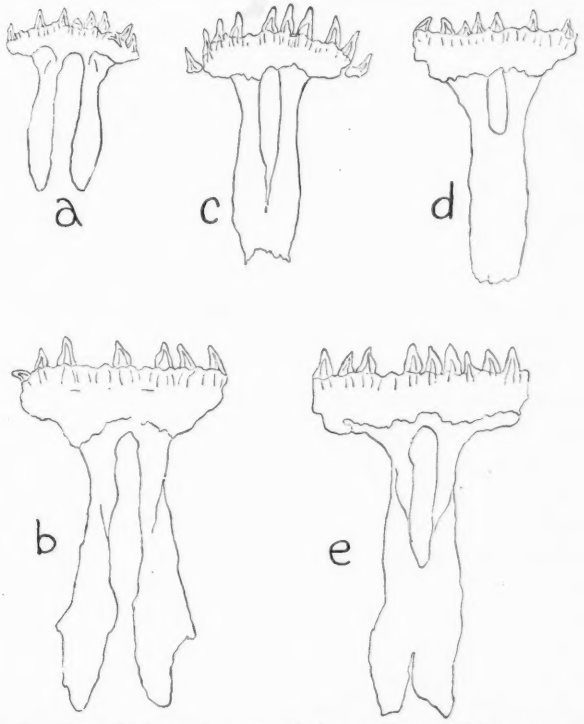

FIG. 1. Ventral views of premaxillaries of *Eurycea bislineata*. × 18. Drawn from dissociated bones stained with alizarine. (a) Usual types as seen in a 27 mm larva; (b) Usual type as seen in an 81 mm adult male; (c) Unusual type found in a 44 mm larva; (d) Unusual type found in a 55.7 mm advanced metamorphic individual; (e) Unusual type found in a 67 mm young adult male.

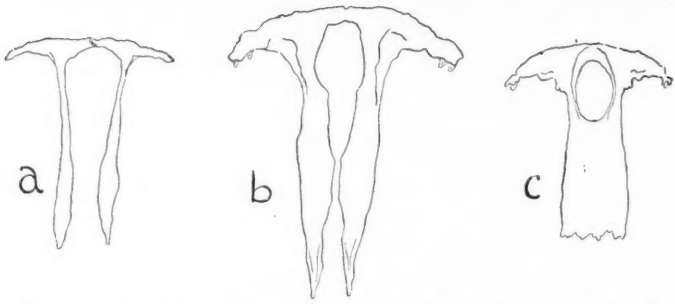

FIG. 2. Dorsal views of premaxillaries of (a) Plethodon, (b) Gyrinophilus (unusual type), and (c) Pseudotriton. × 18. Drawn from *in toto* preparations stained in alizarine and cleared in glycerine, loaned by Dr. E. R. Dunn.

off the skin over the dorsal surface of the snout, thus revealing very satisfactorily against the white background of the surrounding tissues the whole dorsal aspect of the purplish-red premaxillary. Among the 109 specimens thus examined five (or 4.5 per cent.) were found to be of the unusual, more extensively fused type. These, together with controls of approximately the same size and developmental stage, were then further dissected to remove certain muscles and glands, and subjected to partial dissociation and clearing by means of caustic potash and glycerine, in order that the form and relationship of the premaxillary might be worked out and drawn more accurately. In all this work a binocular dissecting microscope was used, and the drawings were made by the use of a Zeichenokular, attached to this.

The five cases, which, together with the controls, are shown in Figure 3, comprise the following sizes and developmental stages:[2]

Typical larval male, length 22 mm.

Incipient premetamorphic female, length 37 mm.

Premetamorphic females, lengths, 46 mm, 51 mm, and 56.6 mm, respectively.

[2] Wilder, I. W., "The Relation of Growth to Metamorphosis in *Eurycea bislineata*," *Jour. Exp. Zool.*, Vol. 40, 1924.

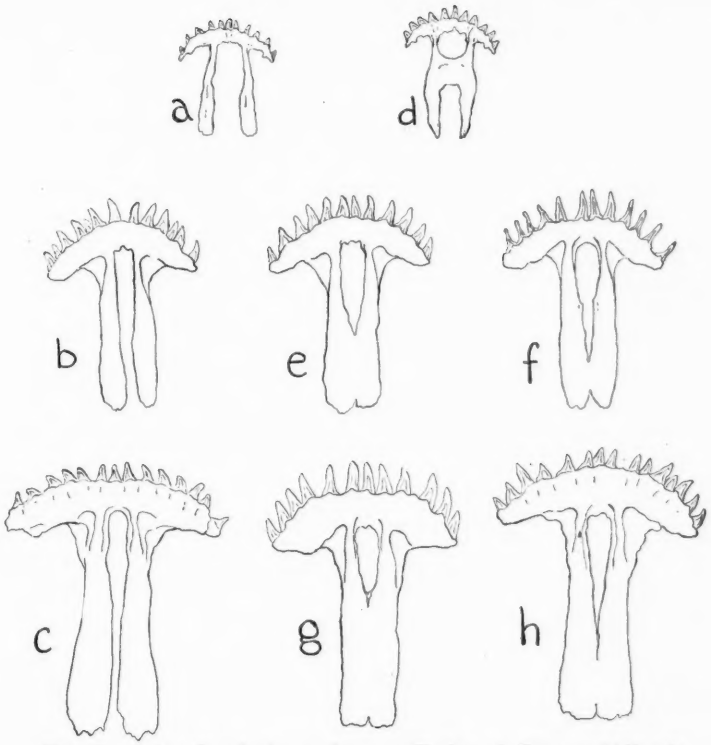

FIG. 3. Antero-dorsal views of premaxillaries of *Eurycea bislineata*. × 18. Stained in alizarine and cleared in glycerine. Usual types: (a) 25 mm typical larval male; (b) 36 mm incipient premetamorphic male; (c) 51 mm premetamorphic female. Unusual type: (d) 22 mm typical larval male; (e) 37 mm incipient premetamorphic female; (f) 46 mm premetamorphic female; (g) 51 mm premetamorphic female; (m) 56.6 mm premetamorphic female.

Comparison of these cases shows a considerable degree of variation in the extent of union, which, however, does not seem to be correlated with the size or the developmental stage of the individual. This is particularly emphasized by the fact that the young larva shows one of the most conspicuously marked types of all, since in this stage, as is shown by the control, the ascending processes are normally so widely separated that their union across

the midline can by no possibility be attributed to a mere chance difference in the extent of ossification leading to the fusion of parts otherwise nearly in contact. That we are dealing here with a distinct variant is further indicated by the fact that the fusion of the ascending processes results in most cases in a somewhat narrower ascending region than that shown by the control. Attention should be called to the fact that examples of the unusual type have been found in both sexes and from two different localities, Western Massachusetts and Long Island, New York.

The premaxillary has never been given an important taxonomic value in the Caudata. While the Proteidae, Cryptobranchidae, Sirenidae, Hynobiidae and Ambystomidae show, so far as has been reported, the primitive paired or unfused type of premaxillary and the Amphiumidae the unpaired or fused type, in the large families of the Salamandridae and the Plethodontidae both types are present. In the latter family, for example, Ensatina, Plethodon, Hemidactylium, Hydromantes and Gyrinophilus are described as presenting the typical primitive paired form (Figure 2, a) while other genera such as Œdipus, Eurycea, Pseudotriton, Batrachoseps and Desmognathus show the unpaired type with varying degrees of fusion from a form like Œdipus, in which only the extreme anterior region is involved, to forms like Pseudotriton and Desmognathus in which the ascending processes are so completely fused as to leave only a fontanelle. It is evident from the data presented in this paper that *Eurycea bislineata* shows a range of variation of the premaxillary which includes these two extremes and thus covers differences which exist in this regard between different genera.

My colleague, Dr. E. R. Dunn, informs me that he has observed occasional cases of fusion of the ascending processes of the premaxillary in other species of Eurycea. He has also brought to my attention an equally significant variation which he has found in the premaxillary of Gyrinophilus (Figure 2, b). In this the simple fused

type is found instead of the paired type supposed to be characteristic of this genus. We have no data as to possible percentage of occurrence of this fused type in Gyrinophilus. This single case, however, emphasizes the probability of a similar variability in the premaxillary in any form, and should be a forcible warning against raising this bony element to the taxonomic importance which Noble[3] gives it when he makes it one of the three distinguishing features of the genus Œdipus: "(1) No prefrontal; (2) no septomaxillary; (3) premaxillae ankylosed only at their extreme anterior ends." The weight which Noble gives to the third point he further emphasizes in his statement, "Œdipina, which on external features one would consider nothing but an elongate Œdipus, differs radically from this genus in its fused premaxillae," apparently, moreover, basing his knowledge on a single specimen of Œdipina to which he refers as "the specimen of *Œdipina uniformis* which I dissected." However this may be, every taxonomist knows that in many instances only a single individual is available and that too often, even when there is an abundance of material at hand, descriptions are based upon either a single or at best a very few individuals.

If the premaxillary, which is one of the oldest and most constant of bones of the vertebrate skull, proves to be so variable within the limits of a single species, one is forced to believe that other bones might be found to be no less so, were a hundred individuals to be examined. Is it not possible that the *range of variation* of such a part may in itself be a matter of much greater significance as indicative of systematic relationships than a single type regarded as a constant character for the genus or species? From the standpoint of the morphologist, certainly, one would wish to urge the examination of larger numbers of individuals with regard to each detail of structure which is to be made use of, as a sound basis for descriptive and taxonomic work.

[3] Noble, G. K., "The Anterior Cranial Elements of Œdipus and Certain other Salamanders," *Bull.* of Am. Mus. of Nat. Hist., Vol. XLIV, 1921.

FURTHER STUDIES OF THE RELATIONSHIPS OF THE STRUCTURAL CHARACTERS OF MAMMALIAN HAIR[1]

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SINCE the writer's preliminary studies of the structural characteristics of the hair-shafts of mammals were made,[2] continued observations have been throwing more and more light upon the relationships of the hair-shaft structures to one another, and to the groups of mammals in which they occur. In the earlier studies it was pointed out that the four structural units of the hair-shafts examined are susceptible of classification upon the basis of form.

The four structural elements of the mammalian hair-shaft (Fig. 1) are: (1) The medulla, or "pith" of the hair, made up of variously shaped and disposed cells or chambers, representing cornified epithelial elements; (2) the cortex, surrounding the medulla, composed of elongated, fusiform, often much-shrunken cells (sometimes referred to as the hair-spindles) coalesced into a rigid,

[1] The writer acknowledges, with gratitude, his indebtedness to the following, for sending him samples of hair: Dr. F. A. Lucas, the late Dr. J. A. Allen, Mr. L. R. Sullivan and Mr. H. Lang, of the American Museum of Natural History in New York; Dr. H. D. Reed, of Cornell University; Dr. Aleš Hrdlička, Dr. G. S. Miller and Dr. N. M. Judd, of the United States National Museum in Washington; Dr. A. K. Fisher, of the Bureau of Biological Survey; Mr. C. G. Potts, of the United States Department of Agriculture; Dr. Chi Ping, of the Southeastern Teachers' College, Nanking, China; Dr. R. L. Ditmars and Dr. W. T. Hornaday, of the New York Zoological Gardens; Dr. F. R. Speck, of the University of Pennsylvania; the Metropolitan Museum of Art, in New York; Dr. T. C. Nelson, of Rutgers College, and Mr. M. W. Meek, of the Meek, Court Co.

[2] Hausman, L. A.: (1) "A micrological investigation of the definitive hair structure of the Monotremata," *Am. Jour. of Anat.*, Sept., 1920, p. 563; (2) "Structural characteristics of the hair of mammals," AM. NAT., Vol. 54, 1920, p. 496; (3) "Mammal fur under the microscope," *Nat. Hist.*, Vol. 20, 1920, p. 434.

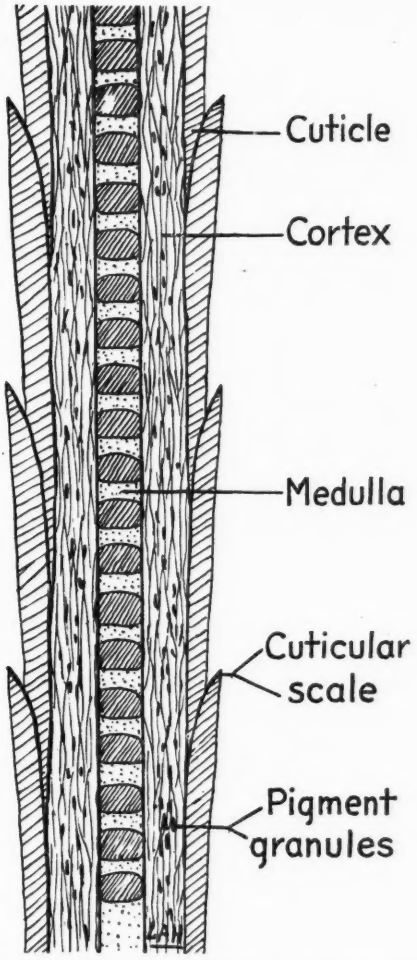


FIG. 1. Optical longitudinal section through a generalized mammal hair of the discontinuous medulla type.

almost homogeneous, hyaline mass; (3) the pigment granules, to which the color of the hair is primarily due (though in some hairs the pigment is diffuse). These granules are distributed within and among the hair-

spindles. Studies which have been[3] and are now being made, of the disposition of the pigment granules, and especially of the patterns which they form in the hair-shaft, give earnest of some interesting correlative data. (4) The cuticle of the hair, which is its outermost integument and which is composed of thin, horny, transparent plates of a multitude of forms.

The cuticular scales of the hair are of two general kinds (Fig. 2), the imbricate and the coronal. Of the

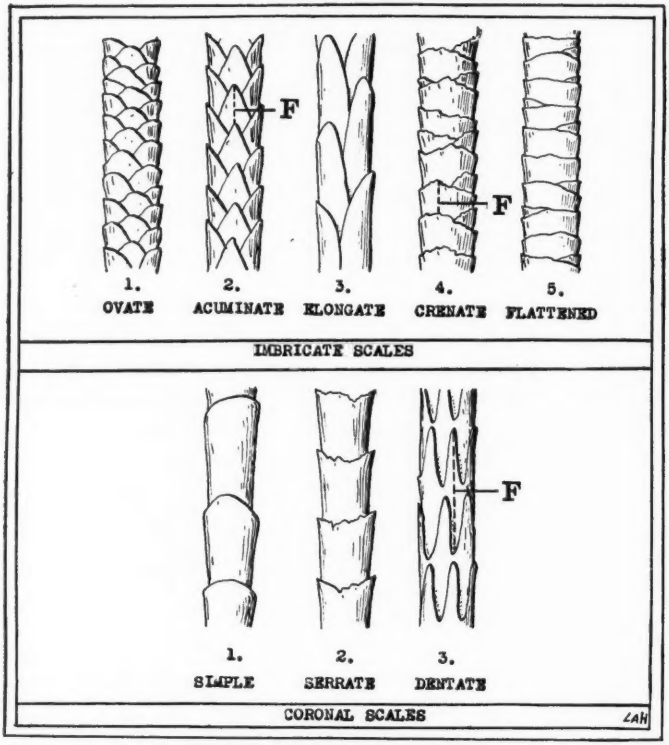


FIG. 2. The eight types of cuticular scales of mammalian hair. F shows how the proximo-distal diameters of the free surface of the scales are measured.

[3] Hausman, L. A., "Hair coloration in animals," *Sci. Mon.*, Vol. 12, Mar., 1921, p. 215.

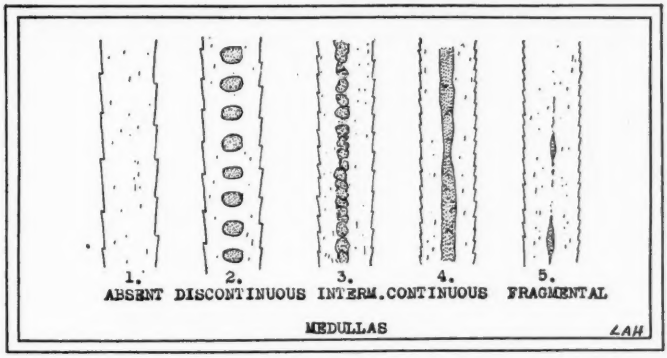


FIG. 3. The five types of medullas of mammalian hair.

former we have recognized five elemental varieties, *viz.*, ovate, acuminate, elongate, crenate and flattened. Of the latter there are three varieties, *viz.*, simple, serrate and dentate. All scale forms which have now been found in the hairs of mammals (in all over 370 species have been examined) are referable, as variations, to these eight categories.

The medullae lend themselves to classification thus (Fig. 3): (1) Absent altogether, (2) discontinuous, as when the medullary cells or chambers are separated, (3) intermediate, with the separate chambers beginning to fuse, (4) continuous, in which the cells are massed into a nearly uniform rod-like structure in the center of the hair-shaft, and (5) fragmental, in which case elongate fragments of the medulla are distributed irregularly along in the axis of the shaft.

In the discussion of the scale and medulla characteristics of hairs which follows, the status of these structures is considered midway between the base and the tip of the hair. The hairs used for comparison were the under or fur-hairs of the specimens, taken from the region of the middle of the dorsum. It was found that among the *Primates,* below the *Hominidae,* the under hair from the dorsum was identical in structure with that of the head.

The nature of the relationship between the hair structure and hair-shaft itself was first suggested to the writer following the examination of the hairs of sixteen species of *Primates,* chosen at random and representing the nine families and ten subfamilies of the order (below the *Hominidae*) as given by Elliot.[4] The first relationship which presented itself was that between the systematic positions of the specimens from which the hairs were taken, and the diameters of the hair shafts. Fig. 4 graphically records this relationship.

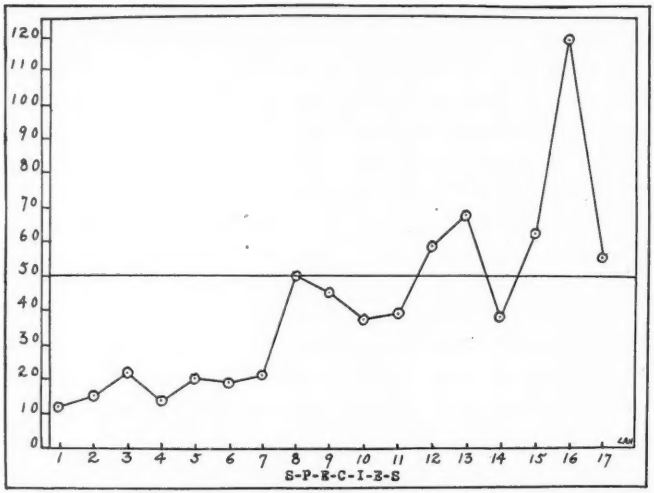


FIG. 4. The relationship between the diameters of the hair shafts of seventeen species of *Primates* and the morphological complexity of the species. Along the ordinate are given the hair shaft diameters in microns, and along the abscissa are enumerated the species in the order of morphological complexity.

It was noted, moreover, that an increase in the diameter of the hair shaft was accompanied by a decrease in the width of the free surface of the cuticular scales, *i.e.*, in its free proximo-distal diameter (F, Fig. 2). A numerical expression of the relation between the free prox-

[4] Elliot, D. G., "A review of the *Primates,*" Monograph, Am. Mus. Nat. Hist., 1912.

imo-distal diameter of the cuticular scales, and the hair-shafts on which they occur was devised, and termed the *scale index*. This is arrived at by dividing the free proximo-distal diameter of the cuticular scales by the diameter of the hair-shaft, and expressing the result in decimal form. Where D is the diameter of the hair-shaft, F the free proximo-distal diameter of the scales, and S the scale index:

$$\frac{D}{F} = S.$$

Computations of the scale indices of the seventeen species of *Primate* hairs mentioned gave the data for plotting the graph shown in Fig. 5.

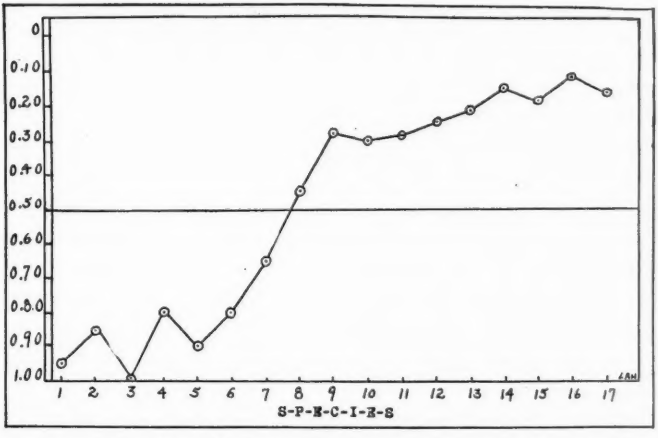


FIG. 5. The relationship between the scale index (see Fig. 2) of the hairs of seventeen species of *Primates*, and the morphological complexity of the species. Along the ordinate are given the scale indices, and along the abscissa are enumerated the species in the order of morphological complexity.

An examination of the medullas of these same specimens of hairs revealed the fact that the increase in the diameter of the hair-shafts was accompanied by a change in medulla form, from the discontinuous type of medulla to the fragmental type, as shown in Fig. 6. The fact that

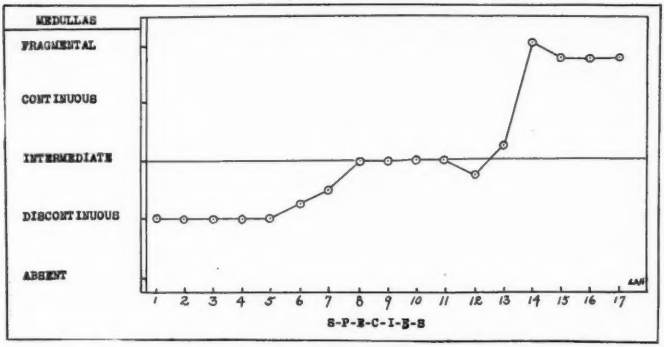


FIG. 6. The relationship between the medulla types (see Fig. 3) in the hairs of seventeen species of *Primates*, and the morphological complexity of the species. Along the ordinate are given the medulla types, and along the abscissa are enumerated the species in the order of morphological complexity.

this comparative study of the hairs of so limited a number of species of mammals, taken quite at random, showed such relationships, indicated that perhaps similar relationships existed among mammals in general, and incited the observer to more inclusive examinations. Accordingly, 190 samples of dorsal under hair, from as many species of mammals (representing all the existing orders except the *Cetacea*) were examined for the status of their scale and medulla elements.

Computations of the scale indices of these hairs and the measurements of their diameters gave the data shown in Fig. 7.

In this figure the diameters of the hair-shafts examined are plotted along the abscissa, and the scale indices along the ordinate. Here again the same relationship between scale index and shaft diameter, as was noted in the *Primate* hairs, became apparent. Since the scale index also denotes, in general, the form of the scale, this relationship between scale index and shaft diameter is interesting and significant, inasmuch as it implies that the forms of the cuticular scales of mammals bear relation, not to the groups to which the species have been assigned, but

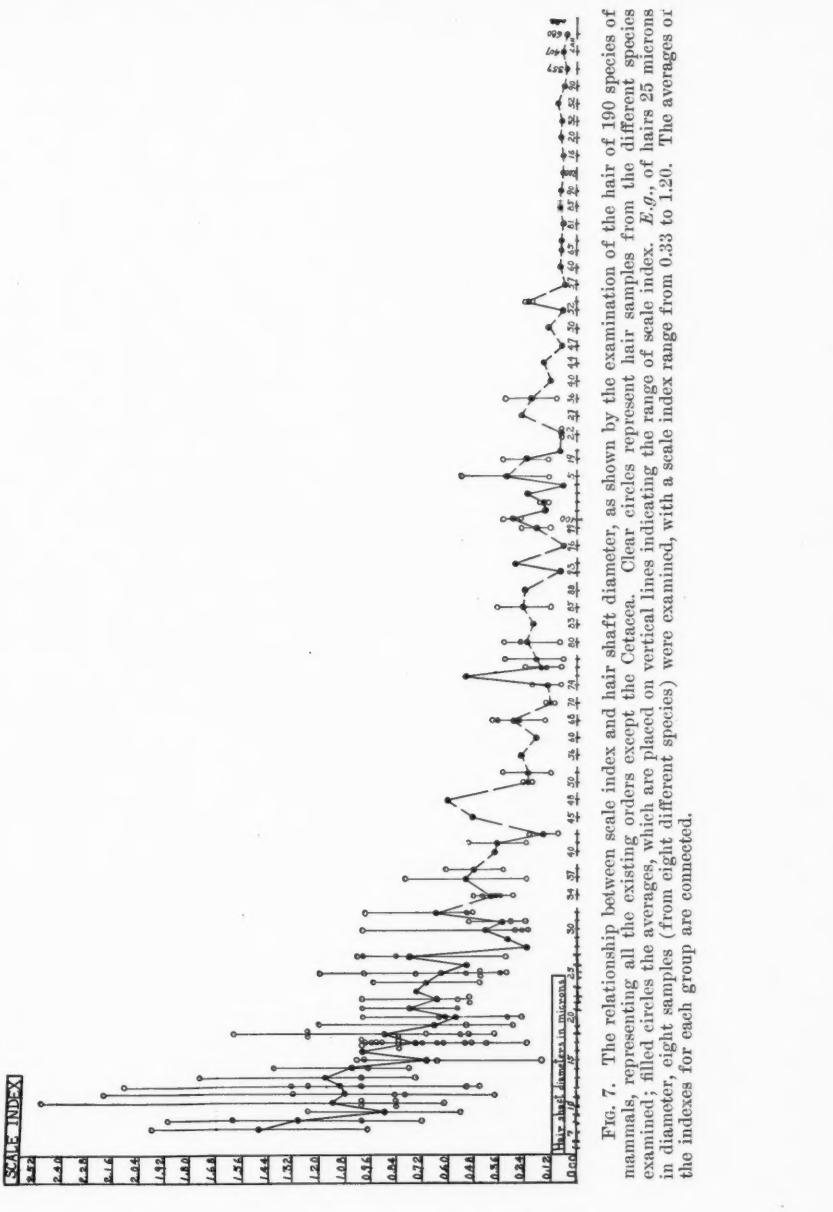


FIG. 7. The relationship between scale index and hair shaft diameter, as shown by the examination of the hair of 190 species of mammals, representing all the existing orders except the Cetacea. Clear circles represent hair samples from the different species examined; filled circles the averages, which are placed on vertical lines indicating the range of scale index. *E.g.*, of hairs 25 microns in diameter, eight samples (from eight different species) were examined, with a scale index range from 0.33 to 1.20. The averages of the indexes for each group are connected.

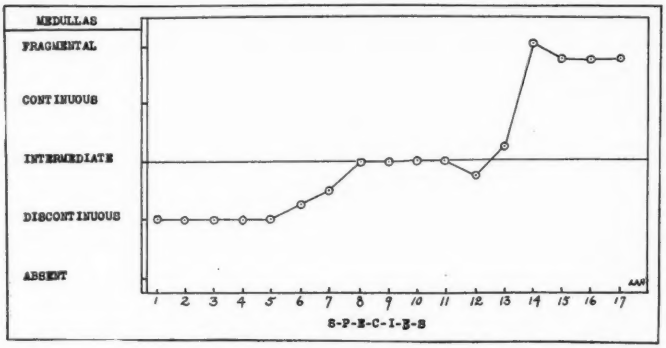


FIG. 6. The relationship between the medulla types (see Fig. 3) in the hairs of seventeen species of *Primates*, and the morphological complexity of the species. Along the ordinate are given the medulla types, and along the abscissa are enumerated the species in the order of morphological complexity.

this comparative study of the hairs of so limited a number of species of mammals, taken quite at random, showed such relationships, indicated that perhaps similar relationships existed among mammals in general, and incited the observer to more inclusive examinations. Accordingly, 190 samples of dorsal under hair, from as many species of mammals (representing all the existing orders except the *Cetacea*) were examined for the status of their scale and medulla elements.

Computations of the scale indices of these hairs and the measurements of their diameters gave the data shown in Fig. 7.

In this figure the diameters of the hair-shafts examined are plotted along the abscissa, and the scale indices along the ordinate. Here again the same relationship between scale index and shaft diameter, as was noted in the *Primate* hairs, became apparent. Since the scale index also denotes, in general, the form of the scale, this relationship between scale index and shaft diameter is interesting and significant, inasmuch as it implies that the forms of the cuticular scales of mammals bear relation, not to the groups to which the species have been assigned, but

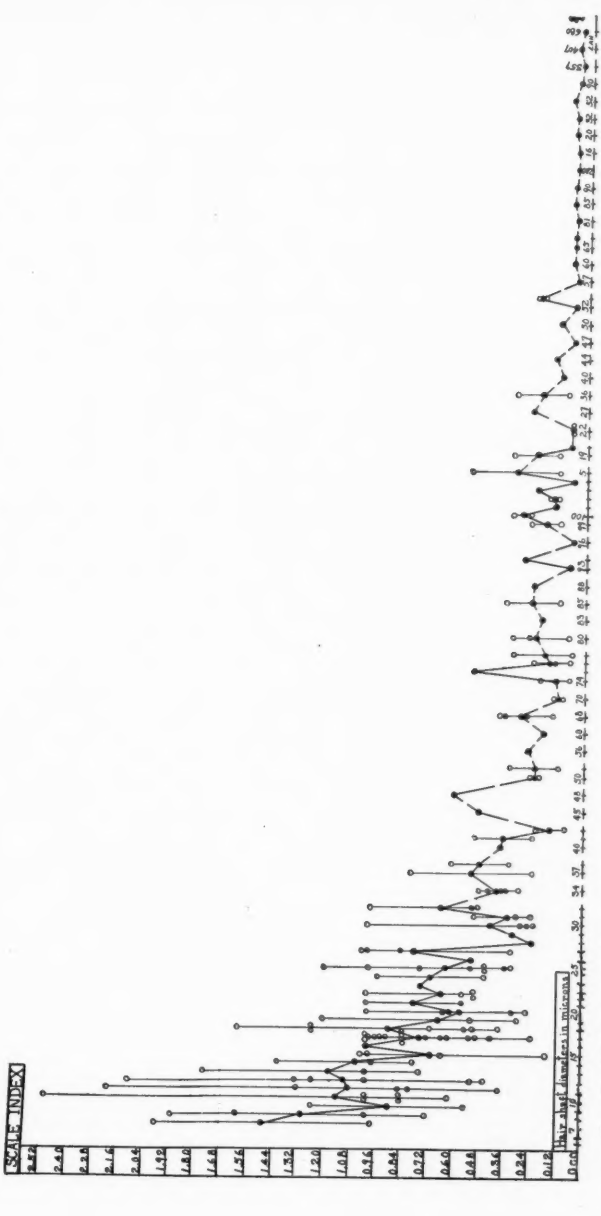


FIG. 7. The relationship between scale index and hair shaft diameter, as shown by the examination of the hair of 190 species of mammals, representing all the existing orders except the Cetacea. Clear circles represent hair samples from the different species examined; filled circles the averages, which are placed on vertical lines indicating the range of scale index. *E.g.*, of hairs 25 microns in diameter, eight samples (from eight different species) were examined, with a scale index range from 0.33 to 1.20. The averages or the indexes for each group are connected.

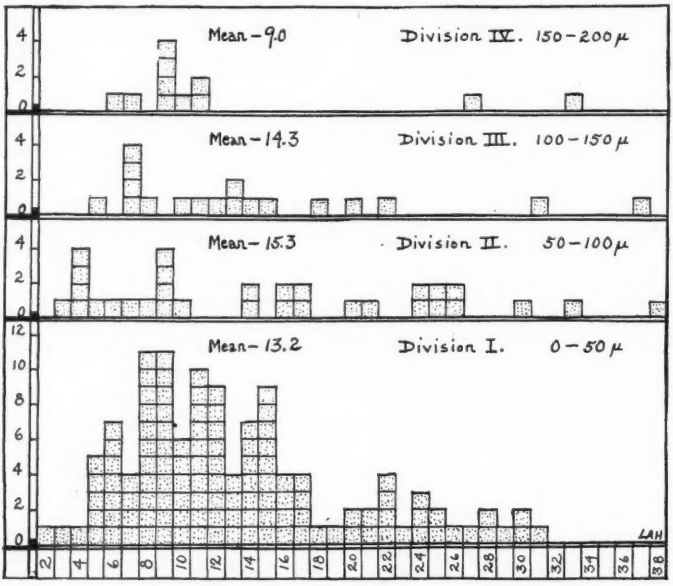


FIG. 8. Lengths of the free proximo-distal diameters of the cuticular scales, plotted against the frequency of occurrence. The micron-length-groups are arranged along the abscissa; the frequency of the occurrence of the examples in each group along the ordinate. The 178 hair-shafts examined (from as many species of mammals) are separated into four groups, or divisions:—Division I contains hair-shafts from 0 to 50 microns in diameter; Division II those from 50 to 100 microns; Division III those from 100 to 150 microns; and Division IV those from 150 to 200 microns. The mean length of scale for each division (*i.e.*, mean proximo-distal diameter).

to the diameter of the hairs which they bear (Plate I). That even one individual may bear upon its body hairs of different and widely separated structural groups was shown in the writer's studies of the hairs of the *Ornithorhynchus anatinus* and the *Tachyglossus hystrix* (1).

Fig. 8[5] shows the frequency of the occurrence of various cuticular scale lengths (*i.e.*, the proximo-distal diameters), in microns, arranged in groups or divisions. The mean lengths of the scales of the four divisions are much

[5] The writer is much indebted to Professor W. J. Crozier, of Rutgers College, for his aid in the preparation of this figure.

alike, and the scale lengths are distributed with similar regularity in each of the divisions.

Next, a survey of the medullas of the hairs of 200 species of mammals, representing all the existing orders except the *Cetacea,* yielded the information contained in Table I, with respect to the relationship between medulla-form and hair-shaft diameter. Five different groups of hairs were recognized, according to the types of medullas they contained. Such a grouping showed that as a rule the finer hairs (*i.e.,* those of small shaft diameter) contained no medullas, or either the discontinuous or intermediate varieties; while the coarser hairs bore the continuous or fragmental varieties (see Fig. 3). Only a meagre number of exceptions to this rule were found, as

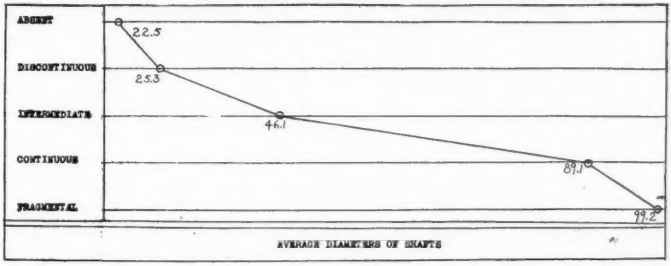


FIG. 9. To show the relation of hair-shaft diameter to type of medulla, a graphic presentation of the material is contained in Table No. 1. Along the ordinate are given the medulla types (see Fig. —), and along the abscissa the average diameters of the hair shafts exhibiting these five different types.

the table shows. Fig. 9 represents in graphic form the results of the study of the distribution of medulla-forms. Here, again, the interest lies in the implication that medulla-form, like scale-form, is related not to natural group, but to hair-shaft diameter. Medulla-form may vary in the hairs even of an individual, as in the case of the *Ornithorhynchus anatinus* and *Tachyglossus hystrix* (1).

An interesting fact brought out by the study of scale types is that with the finer hairs there is a much greater range in the scale index than with the coarser. That is,

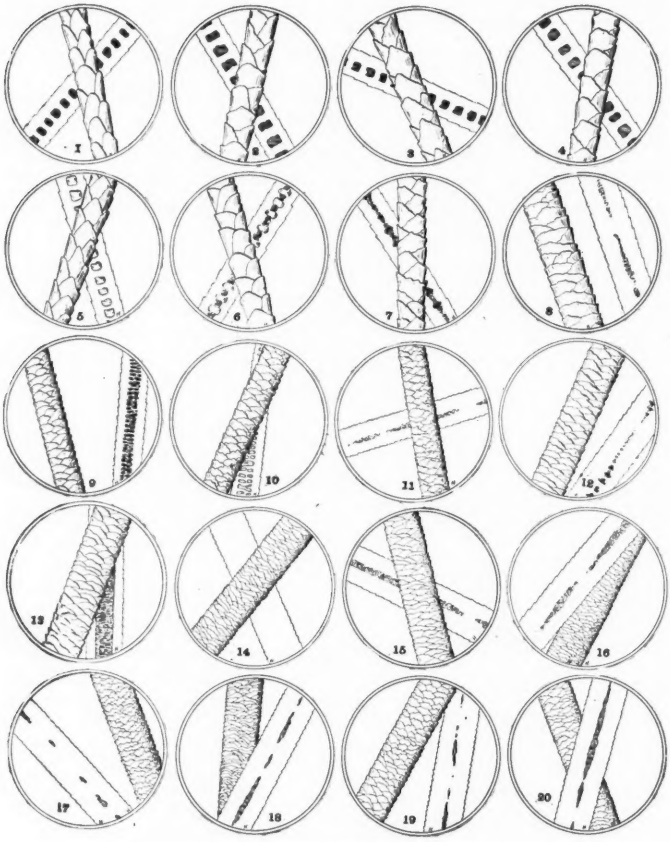


PLATE I

The first sixteen specimens are of dorsal hair, taken from as many species of *Primates*, arranged in the order of the morphological complexity, and representing the nine families and ten subfamilies of the order as given by Elliot (footnote 4). The last four specimens are of human head-hair, from individuals representing four races of mankind. Each figure depicts the portions of two hair-shafts; one prepared to show the cuticular scales, the other to show the medulla. The micrographs were made from the middle portion of the hair (*i.e.*, midway from the base to the tip of the hair-shaft). The numbers following the name of each species in the list below are the diameters of the hair-shafts in microns.

fine hairs may possess relatively very large scales (as in the Golden Mole, *Amblysomus corriae*), or relatively small ones (as in the Flying Squirrel, *Sciuropterus volucella*); while, with the coarser hairs (those, *e.g.*, of a diameter greater than 50 microns) the scales are relatively uniformly small. A large scale index (above 1.80, let us say) usually denotes the elongate or acuminate variety of imbricate scale; or the coronal scale. A medium scale index (about 0.50 to 0.75) denotes, as a rule, some of the larger varieties of the ovate, crenate or flattened scale; while an index below 0.35 usually denotes the smaller variety of either the crenate or the flattened forms (Figs. 1 to 20, Plate I).

FIG. 1. Aye aye (*Chiromys madagascariensis*)—11
FIG. 2. Tarsier (*Tarsius fuscus*)—15
FIG. 3. Potto (*Perodicticus ibeanus*)—22
FIG. 4. Galago (*Galago demidoffi*)—14
FIG. 5. Ruffed Lemur (*Lemur varius*)—20
FIG. 6. Sifaka (*Propithecus coronatus*)—18
FIG. 7. Guenon (*Cercopithecus patas*)—21
FIG. 8. Howler (*Alouatta palliata inconsonans*)—50
FIG. 9. Squirrel Monkey (*Chrysothrix sciurea*)—45
FIG. 10. Aotus (*Aotus senex*)—37
FIG. 11. Geoffroy's Spider Monkey (*Ateles geoffroyi*)—38
FIG. 12. Heuglin's Baboon (*Papio doguera heuglinii*)—58
FIG. 13. Proboscis Monkey (*Nasalis larvatus*)—67
FIG. 14. Hoolock Gibbon (*Hylobates hoolock*)—37
FIG. 15. Gorilla (*Gorilla gorilla*)—62
FIG. 16. Schweinfurth's Chimpanzee (*Pan schweinfurthii*)—118
FIG. 17. Bushman of South Africa (Negro Race)
FIG. 18. Chinese (Mongolian Race)
FIG. 19. Peruvian mummy, of cir. 200 A. D. (American Race) } 50 to 65
FIG. 20. English (Caucasian Race)

A microscopic study of some fifty-odd specimens of human head-hair, representing all the existing grand divisions of the races of mankind, showed that there were no very well-marked variations in either scale- or medulla-form.[6] In general, the coarser the hair, the finer

[6] Further work, tentatively outlined, and covering a greater range of samples, must be done before any definite pronouncement can be ventured regarding the scale and medulla relationships in the hairs of the *Hominidae*. There seem to be, however, considerable variations in the pigment granules

the scalation, as might be inferred from the study of *Primate* hair, below the *Hominidae,* just made. The lanugo from a foetus of North Mediterranean stock, of about seven months, averaged about 15 microns in hair-shaft diameter, and exhibited scales of the flattened imbricate type (Fig. 2), whose index was 0.33. The hair, consequently, resembled that of the Howler Monkey (Fig. 8, Plate I).

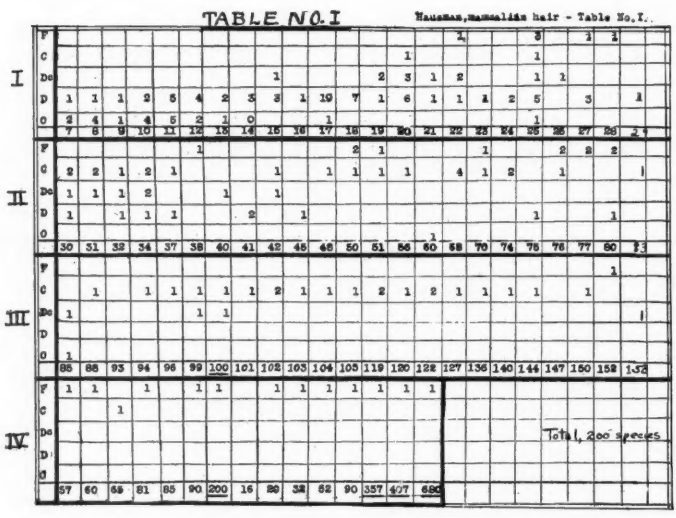


TABLE NO. I

Showing the diameters of the hair-shafts examined, in microns (from 7 to 680), below. The column above each figure enumerates the "species of hair" examined, and the letters at the left indicate the five types of medullas encountered. *E.g.,* of hairs 10 microns in diameter, 6 species were examined, 4 of which possessed no medullas and 2 of which possessed the discontinuous type.

O—no medulla.
D—discontinuous medulla.
DC—intermediate medulla.
C—continuous medulla.
F—fragmental medulla.

in the hairs of the various races. The granules vary in size, in color value and depth, and particularly in the shapes of the patterns which they form in the cortex of the hair. Some preliminary studies (see footnote 3) have been made of these granules, and the observations are being continued in the splendid series of samples of human head-hair, of various races, given to the writer by Dr. Aleš Hdrlička, of the National Museum in Washington.

It is rather remarkable, although variations are not uncommon, to find a hair-shaft of this meagre diameter clothed with scales of the flattened type, and of so low an index. The majority of hairs between 10 and 20 microns of the mammals, below the *Hominidae,* that were examined possessed ovate scales, with an average index of 0.90. As a rule, the flattened type of scale, of the index presented by this lanugo, was encountered generally upon hairs of from 50 to 100 microns in diameter. No medulla could be discerned.

Summary

In the specimens of mammal hairs examined:

(1) Scale-form (as expressed by the scale index, a mathematical expression of the relationship between the free proximo-distal diameter of the scales and the diameter of the hair-shaft) bore relation not to the natural group to which any given species belonged, but to the diameter of the hair-shaft. In other words, the coarser the hair the finer the scales, or the magnitudes of the free proximo-distal diameters of the cuticular scales and the diameters of the hair-shafts varied inversely.

(2) The medulla-form varied with the diameters of the hair-shafts, and not with natural groups of mammals, in a definite way.

(3) Hence, given the diameter of a hair-shaft, and regardless of the species from which it was derived, it should be possible to locate it in its proper medulla-form, or scale-form group, approximately.

(4) It is inferred that the relationships between scale-form (as expressed by the scale index), medulla-form and hair-shaft diameter, which have been found in the series of samples examined in this study, obtain also among mammals in general.

(5) From the results of previous studies of mammal hairs, as well as from added results from this present one, it can still be said, however, that specific differences of sufficient appreciable magnitude exist, commonly, to aid in identifying the species of mammal from which a given hair sample was obtained.

SHORTER ARTICLES AND DISCUSSION

THE SIMILARITY OF AGE VITALITY IN INVERTEBRATES AND MAN BASED ON PROFESSOR RAYMOND PEARL'S DATA

DURING recent years Professor Raymond Pearl has published a considerable amount of data regarding the length of life of flies and has constructed life tables from them.[1] The resemblance of these life tables to those of man suggested that it might be worth while applying some further tests to his figures.

Some time ago I devised and published the series of formulae by which the expectation of life in man could be easily calculated by means of the use of a standard population, if the death-rates at certain groups of ages were known. These formulae were based upon the numerous life tables constructed in England and Wales ranging from the life table referring to the very unhealthy district of Manchester Township to that of the healthiest of the country districts. Later, the method was much improved. The standard population now chosen decreases in a simple arithmetical progression as age increases, as was long ago suggested by De Moivre. In practice, the population between 0–5 years is assumed to be 16,000, that between 5–10 years 15,000 and so on. Thus between 25 and 35 years it numbers 21,000, between 35 and 45 years 17,000, while above 75 years it is reduced to 1,000. The death-rates at the groups of ages just described, obtained from the statistics, are applied to this population, and the number of deaths that would occur in this standard population with these death-rates above each age obtained. From these values, the expectation at each age can be at once calculated. The method will be better understood by considering an example. The example selected refers to the long-winged male *Drosophila*, for which if its 100 days of life be taken equal to a 100 years of life in man, the distribution of survivors falls well within the range of human variation. The death-rates were calculated for the age periods 0–5 days, five-daily periods after that to 25 days, thereafter by ten-daily periods to 75 days and lastly the death-rate above 75 days.

[1] Pearl, R., "Medical Biometry and Statistics," p. 177.

The method of working is shown in Table I. In the first column the standard population is given; in the second column the absolute death-rates as calculated; in the third column the sums of the deaths above each age—thus the lowest figure in the column is obtained by multiplying the death-rate in the second column by 1,000. The next figure above it in the column is obtained by adding this to 5,000 multiplied by the death-rate between 65 and 75 years, the figure above by adding to this sum 9,000, multiplied by the death-rate between 55 and 65 years, and so on to the top of the column. Denoting these sums by D_x and the expectations by E_x we have the relationship,

$$\frac{1000}{E_x} = m D_x + c$$

or

$$E_x = \frac{1000}{m D_x + c}$$

The two constants m and c calculated by means of the five best life tables for England and Wales for each age and for both sexes are given in Table II. The figures in column 4 which give the expectations are obtained by the use of the formula and the values of the constants m and c. These are now to be compared with the figures in column 5, which give the expectations of life as calculated by Professor Pearl in the usual manner. It will be noticed that the correspondence is very close except at birth, when the formula derived from man gives 5 days less life

TABLE I

ILLUSTRATION OF THE METHOD

Age	Standard population	Death-rates	Number of deaths D_x	Age	Expectation calculated E_x	Expectation actual
0–5.....	16,000	.0094	3694.5	0	36.0	41.0
5–10.....	15,000	.0112	3544.1	5	39.1	38.5
10–15.....	14,000	.0107	3376.1	10	35.2	35.4
15–20.....	13,000	.0135	3226.3	15	32.5	32.2
20–25.....	12,000	.0147	3019.6	20	29.6	29.1
25–35.....	21,000	.0180	2843.2	25	26.5	26.2
35–45.....	17,000	.0331	2465.2	35	20.5	20.7
45–55.....	13,000	.0513	1902.5	45	15.9	16.1
55–65.....	9,000	.0679	1238.2	55	12.2	12.4
65–75.....	5,000	.0867	582.0	65	9.4	9.5
75 + ...	1,000	.1370	132.0	75	7.4	7.3

than the experiments. This is to be expected, as there is no infantile mortality among the *Drosophila*.

The subject has been extended and applied to others of Professor Pearl's observations. The comparison of the calculations made by the two methods is shown in Table III. Very close correspondence is shown to exist for the long-winged *Drosophila* of the female sex. Dr. Pearl also gives a life table of a rotifer Proales reduced from the data of Dr. Bessie Noyes, adjusting the life of the worm, which is about 10 days, to the 100 years of human life. When the deaths in a standard population are calculated and the formulae applied in the method described, again a close correspondence is obtained.

With regard to a wild *Drosophila*, which has a shorter life extending at most to about 75 days, before applying the method, the data have been adjusted to correspond to a longer life, three days of the flies' life corresponding with four years of that of man. It will be seen from the table that again the fit is exceedingly close. It thus seems that for certain invertebrates the law of age-ing which is derived from man can be applied, though these organisms functionate in a quite different manner.

The question now arises: Is this a universal phenomenon or not? This can only partly be answered from Professor Pearl's data. When observations on the short-winged *Drosophila* and on one of the mutations[2] are examined, not so close a corre-

TABLE II

GIVING THE CONSTANTS REQUIRED TO CALCULATE THE LIFE TABLE DEATH-RATES FROM THE NUMBER OF DEATHS

	Males		*Females*	
Age	m	c	m	c
0–5	0.0047986	10.052	0.0046168	10.604
5–10	0.0036317	12.756	0.0039159	12.460
10–15	0.0044503	13.210	0.0043761	13.395
15–20	0.0050789	14.303	0.0052281	14.202
20–25	0.0061455	15.254	0.0063082	15.132
25–35	0.0076268	16.204	0.0077535	16.154
35–45	0.012322	18.546	0.012239	18.759
45–55	0.021530	22.017	0.020931	22.606
55–65	0.044486	26.720	0.043032	27.667
65–75	0.127236	31.954	0.121408	34.123
75 +	1.003635	2.823	0.876036	20.901

[2] Gonzalez, Bienvenido Maria, "Experimental studies on the duration of life," AMER. NAT., Vol. LVII, No. 651, p. 296.

TABLE III

EXPECTATIONS DEDUCED FROM THE DATA (A), AND CALCULATED FROM THE FORMULA OF TABLE II (B)

	Long winged *Drosophila*[3]				Rotifer *Proales*[4]		Wild *Drosophila*[5] Both sexes		Mutation[6] bpr asp		Short-winged *Drosophila*[7] Females		Manchester Township 1881–90 Males		England and Wales, last quarter, 1918 Females	
	Males		Females													
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
0	41.0	36.0														
5	38.5	39.1	35.6	35.1	67.9	65.8	34.8	35.7			14.0	16.4	40.53	40.46		
10	35.4	35.2	32.4	32.8	62.9	62.1	31.6	32.5	17.7	19.5	13.0	15.6	37.47	36.62	38.4	42.8
15	32.2	32.5	29.2	29.5	57.9	56.5	28.7	29.8	16.6	18.3	12.1	14.4	33.56	33.24	35.0	39.2
20	29.1	30.3	26.2	26.5	52.9	51.6	26.5	27.3	15.1	16.6	11.3	13.4	29.61	29.61	32.3	36.1
25	26.2	26.5	23.3	23.8	48.0	47.1	23.5	24.3	14.2	15.2	10.7	12.4	26.00	26.11	30.4	33.5
35	20.7	20.5	18.3	18.6	38.3	37.7	18.5	18.7	12.5	12.6	9.7	9.6	20.01	20.09	28.9	29.5
45	16.1	15.9	14.3	14.5	29.4	28.7	12.8	13.1	10.3	10.7	8.7	9.1	14.93	15.17	23.2	23.4
55	12.4	12.2	11.5	11.2	21.1	20.7	10.0	9.9	8.5	8.6	7.6	7.5	10.96	10.92	17.1	17.2
65	9.5	9.4	8.7	8.2	14.8	14.0	8.1	7.5	6.1	5.8	6.2	5.7	7.48	7.53	11.1	11.4
75	7.3	6.5	6.8	6.0	9.7	9.4	7.4	7.2	5.2	5.0	4.7	4.3	4.74	4.65	6.7	7.0

In this table A denotes the actual figures, B those obtained by the formulae.

The age in the first column is in all cases the corresponding human age.

[3] Pearl, R., and Parker, S. L., "Experimental studies on the duration of life," AMER. NAT., Vol. LV, No. 641, p. 494.

[4] Pearl, R., ——— ———

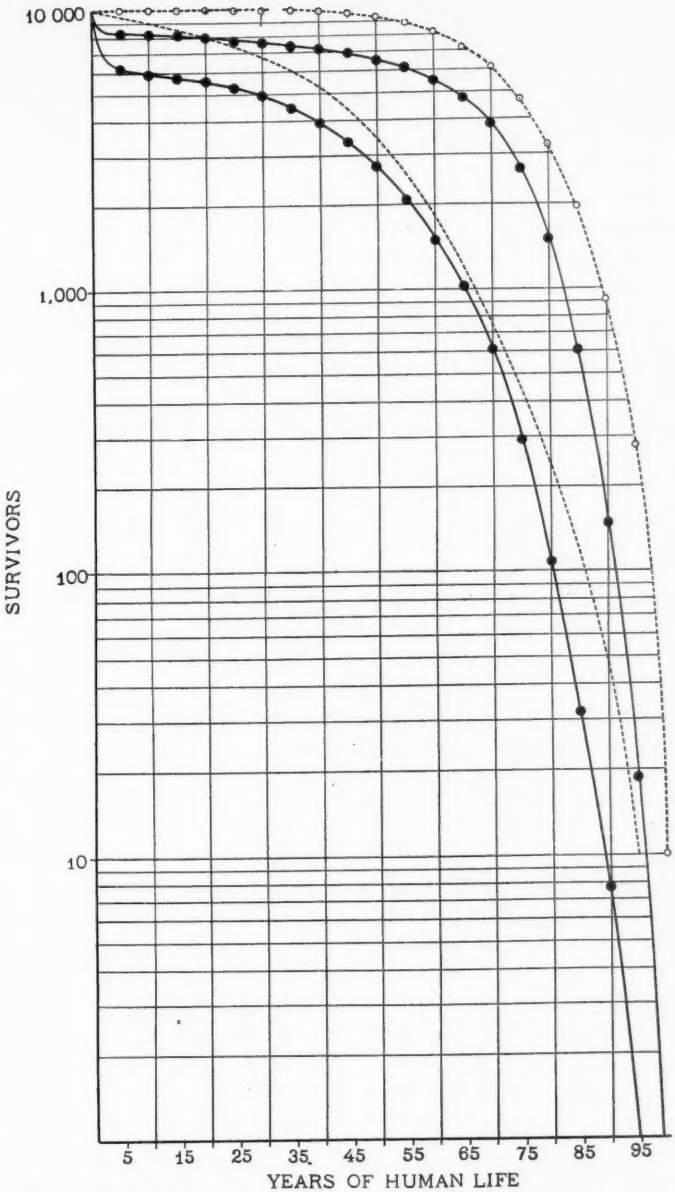
[5] Gonzalez, B. M., "Experimental studies on the duration of life," AMER. NAT., Vol. LVII, No. 651, p. 296.

[6] *Ibid.*, p. 303.

[7] Pearl, R., and Parker, S. L., "Experimental studies on the duration of life," AMER. NAT., Vol. LV, No. 641, p. 497.

spondence is found. The respective results for these are given in columns 11 and 12 and columns 9 and 10 of Table III. It will be seen that above the age corresponding to 35 years the fit is very close and that below this age the formulae based on human life tables in the case of the short-winged *Drosophila* and of the mutations give about one and one half to two days greater expectation. The formula, therefore, fails in this region. A possible reason for this will appear on considering the diagram. In this diagram, the logarithms of the number of survivors in two of the most diverse English life tables, namely, the healthy districts life table for 1881–90 and Manchester Township for the same period, are shown by continuous lines. One dotted line shows the number of survivors for the long-winged *Drosophila*. This curve lies in the zone for which the formulae hold except in the first few days of life. With regard to the rotifer *Proales*, the curve lies outside the curve of the healthy district life table. In this instance an extrapolation is justified by its results, which perhaps might be expected from the close resemblance of the form of the two curves. With regard, however, to the short-winged *Drosophila*, the formulae only hold above the age corresponding to 35 years in man and below that the formulae give an excess. It can not be said that this necessarily means that the laws of life which apply to man may not apply even here because there is no evidence of what occurs with regard to men living in more unhealthy conditions than the Manchester Township. It might be possible that the hyperbolas of the formulae no longer hold beyond the Manchester limit and that a term depending on the second power of the deaths would require to be added. This, however, does not seem likely. The Manchester Township life table was not used in the calculation of the formulae, yet, as may be seen in columns 13 and 14 of Table III, the correspondence between the values of the expectations given in the life table and those calculated by the formulae are very close. An extrapolation ranging considerably beyond the data on which the formulae were calculated is thus found to be justified by the results.

I would suggest that it is possible that some epidemic prevailing among the shorter-winged flies gives rise to the high death-rates between the period corresponding to 10 and 35 years in man. The form of the curve of survivors given by Professor Pearl suggests this. In the last quarter of 1918, a great epidemic



This diagram gives the logarithms of the number of survivors at each age for man and two invertebrates. The upper continuous line refers to the healthy districts of England, 1881–1890, the lower line to the Township of Manchester for the same 10 years. Nearly all the life tables regarding England lie within the zone delimited. The dotted line which lies almost wholly within this zone refers to the long-winged *Drosophila* and the dotted line lying outside refers to the rotifer *Proales*.

of influenza occurred in which the chief excess in mortality was found to be between the ages of 15 and 35 years. Making a life table for the female sex, as on account of the war, the male population can not be estimated, it is found that exactly the same discrepancy between the two sets of calculations found above is obtained. In columns 15 and 16 of Table III, the expectations found by direct calculation are compared with those given by the formulae. Again it is seen that above 35 years of age the concordance is very close, while below this direct calculation gives expectations of life three to four years less than those given by the formulae, corresponding exactly to the one and one half to two days' difference found in the case of the short-winged *Drosophila*.

JOHN BROWNLEE

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

NOTE ON DR. JOHN BROWNLEE'S PAPER ON AGE VITALITY[1]

IT is a great satisfaction to have the independent confirmation, reached by a somewhat different method, which Dr. Brownlee's paper affords, of the results and conclusions regarding the fundamental similarity of the laws of mortality in *Drosophila* and man, which have been published from this laboratory at various times during the past two years.[2] We have shown by the employment of a method somewhat simpler, but, so far as I can see, in no respect less precise than that of Dr. Brownlee that when the biologically equivalent life spans of man and wild type *Drosophila* (our Line 107, cf. Pearl and Parker, *loc. cit.*, 1924) are compared, age being measured in centiles of the life span, the life table constants for the two cases became so similar as to be practically identical. We have used for the comparison the survivorship or l_x function of the life table. Dr. Brownlee uses chiefly the expectation of life function e_x. But as there is, from the mathematical nature of the case, a complete and perfect correlation between the corresponding l_x's and the e_x's of the same life

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 105.

[2] *Cf.*, particularly Pearl, R., AMER. NAT., Vol. 56, pp. 398–405, 1922; Pearl and Doering, *Science*, Vol. 57, pp. 209–212, 1923; Pearl, R., *Poultry Science*, Vol. 3, pp. 1–10, 1923; Pearl and Parker, AMER. NAT., Vol. 58, pp. 71–82, 1924.

table, since both are fundamentally derived from the specific death-rates (q_x's), it makes no particular difference which of the derivative functions one uses as the basis of comparison. It in no wise more cogently or completely demonstrates the fundamental similarity of the laws of mortality in *Drosophila* and man to show that the e_x's are in close agreement in the two cases than to show that the l_x's are. What Dr. Brownlee's paper shows that our previous work does not is that his particular method of deriving e_x from a knowledge of d_x in the construction of a life table is just as applicable to mortality data for *Drosophila* as to those for man.

The *essential* point in the methodology of all such comparisons is, of course, the method used to put total life spans which in absolute time duration are widely different upon an equivalent basis. I venture to suggest that all Dr. Brownlee's comparisons would have been quantitatively improved if he had used a somewhat less rough and ready approximation in establishing this datum than the one he did use. Furthermore, there appears to be no biological justification for his assumption that the imaginal life of *Drosophila* and the total postnatal life of man are equivalent.

Dr. Brownlee's suggestion that the widely different form of the life curve of flies bearing the mutant gene *vestigial* from that of either normal wild type *Drosophila* or man is due to an epidemic mortality in vestigials, seems improbable for several reasons. In the first place direct observation of the vestigial flies themselves, in the process of giving rise to their characteristic mortality curve, affords no evidence that they die of an epidemic or indeed any infectious disease. In the second place, we have shown[3] that the characteristic vestigial life curve reappears as extracted F_2 vestigials from a cross of wild type and vestigial flies, precisely as though it were a character inherited in a typical Mendelian manner. It would be difficult to suppose that an epidemic disease followed the gene for vestigial around through this complicated genetic pathway. In the third place, we have shown[4] that under starvation conditions the life curves of vestigial and wild-type flies become practically identical, and like those for the fed wild-type flies, when age is measured in

[3] Pearl, Parker and Gonzalez, AMER. NAT., Vol. 57, pp. 153–192, 1923.

[4] Pearl and Parker, AMER. NAT., Vol. 58, pp. 193–218, 1924; Pearl, R., *Nature*, June 14, 1924.

centiles of the life spans in the two cases. Finally, we have shown, in experiments as yet unpublished, that under at least two different sets of feeding conditions (not starvation) wild type and vestigial life curves can be made nearly identical and of the wild-type (also human) form rather than that of the characteristic vestigial curve on standard food. Altogether, it seems probable that other factors than epidemic disease are responsible for the form of life curve characteristic of vestigial flies on standard food.

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A CASE OF MATERNAL INHERITANCE IN DROSOPHILA

AN increased interest in cases of so-called "maternal inheritance" is evidenced by the recent appearance of several papers. Among these are Uda's article[1] questioning to some extent the frequently cited results of Toyama and Tanaka, the paper of Sturtevant[2] interpreting in terms of maternal inheritance the data of Boycott and Diver on the inheritance of direction of coiling in Limnaea and the general paper of Morgan[3] on the heredity of embryonic characters. It was therefore believed that it might be of value to publish at this stage a preliminary note concerning a peculiar lethal recently arisen whose expression apparently is dependent upon the genetic make-up of the mother. A *bona-fide* case of maternal inheritance in Drosophila, besides being of general value, would offer exceptional opportunity in the way of analysis.*

The mutant appeared in slightly inbred material of *Drosophila melanogaster* which was concerned in an experiment designed to measure the rate of origin of lethal factors in the X-chromosome. The original culture produced one female and 96 males. This greatly decreased proportion of females in the lethal strain is

[1] Uda, Hajime, 1923, *Genetics*, 8, pp. 322–335.

[2] Sturtevant, A. H., 1923, *Science*, LVIII, pp. 269–270.

[3] Morgan, T. H., 1924, *Scientific Monthly*, XVIII, pp. 5–17.

* Since the above was written an article by Warren has appeared which indicates that the size of the egg of *Drosophila melanogaster* depends upon the genetic constitution of the mother rather than upon the constitution of the zygote itself. This is to be expected since the size of the egg is said to be fixed before the entrance of the spermatozoon. Warren, Don C., 1924, *Genetics*, 9, pp. 41–69.

typical; however, there is some variation in the proportion, for although many cultures produce very few females or none, in others the sex ratio approaches equality. The type of sex ratio in which the females are decidedly less numerous is extremely uncommon in Drosophila, for departures from equality in the sex ratio of these flies are usually due to an elimination of males by lethal or semi-lethal genes in the X-chromosomes. However, 31.5° C. decreases the proportion of females.[4] Moreover, sex-limited mutations, *e.g.,* truncate, are known, the expression of which is more extreme in the female than in the male and which may have a corresponding differential effect on the viability of the two sexes. A striking example of this type of mutation was reported by D. H. Thompson[5] at the 1920 meeting of the American Society of Zoologists. A sex-limited, sex-linked recessive lethal had been found that killed all females homozygous for it and caused no lethal effect in the males, but an erect position of the wings. A different mechanism apparently was at work in the culture reported in 1910 by Quackenbush;[6] this gave 135 males and no females, a ratio much like those given in the present case. This family was not further analyzed, but the results of Sturtevant[7] on the crosses between *D. simulans* and *D. melanogaster* make it practically certain that the sex ratio obtained by Quackenbush was due to a cross between these two species, which were at that time not distinguished. The cross of *D. simulans* female by *D. melanogaster* male—but not the reciprocal cross—gives a greatly lowered proportion of females. As will be seen from the following account, the results of outcrosses of the lethal-bearing strain in the present case have features in common with those of this species cross.

The mode of inheritance of the new lethal is of more interest than the reduced proportion of females produced. The effect is transmitted by both sexes. If females from the lethal strain are crossed to males of other stocks, the offspring may give the lethal ratio; but if the reciprocal cross of a lethal-bearing male by a female of another stock is made, the immediate offspring do not give a lethal ratio. The crosses may be so arranged that the progenies have in the two cases the same genetic constitution;

[4] Mann, M. C., 1923, *Jour. Exp. Zool.,* 38, pp. 213–244.

[5] Thompson, D. H., 1921, *Anat. Rec.,* 20, p. 215; an abstract.

[6] Quackenbush, L. S., 1910, *Science,* XXXII, pp. 183–185.

[7] Sturtevant, A. H., 1920, *Genetics,* 5, pp. 488–500.

the only difference being, then, that when the lethal is introduced from the mother most of the daughters die, but when it is introduced from the father they live. In other words, whether a given female lives or dies depends not upon her own genetic composition, aside from the fact of her being a female, but upon that of her mother alone. These crosses as well as others, all to be reported in detail later, indicate "maternal inheritance" of the lethal, as the term is used by Morgan, Sturtevant and others. It is obvious that the greatest difference between the female zygotes of the two crosses lies in the origin of their cytoplasm. The cytoplasm of the eggs from the female of the lethal strain has been so affected (probably before maturation, since such females apparently must be homozygous to give the effect) that the combination of lethal cytoplasm plus two X-chromosomes plus the autosomes is very much less viable than the combination of non-lethal cytoplasm plus two X-chromosomes plus the autosomes, or than lethal (or non-lethal) cytoplasm plus X plus Y plus the autosomes. Whether the Y of the male, whose sisters die, is responsible *per se* for his survival can not at present be definitely stated. That the majority of the female zygotes from a mother of the lethal strain have actually died (or have not been formed), instead of having been "transformed" into males, is suggested by the absence of any intersexual manifestations; it is possibly also indicated by the low yields from culture bottles giving a lethal count. This problem and others are being studied by cytological methods.

When the lethal is crossed to curly stock (the curly gene is a second-chromosomal dominant with a recessive lethal effect, and is linked to certain non-crossover genes[8]) and when the offspring are inbred, the curly females of succeeding generations, irrespective of the origin of their cytoplasm, never give lethal ratios among their immediate offspring, but the non-curly females may do so. That is to say, the hereditary base of the lethal effect involves at least one recessive gene, and this gene is located in the second chromosome. There is, of course, no discrepancy between the fact that specific chromosomal genes are responsible for the effect and the implication that these genes leave their imprint upon the cytoplasm of the individual before the maturation of the egg giving rise to that individual.

[8] Ward, Lenore, 1923, *Genetics*, 8, pp. 276–300.

The theoretical application of the work to the problem of species incompatibilities may briefly be mentioned. The action of the lethal, in killing all or practically all the females of certain cultures, prevents the reproduction of those individuals. But of greater importance in this connection is the situation as regards the mothers of the dead females; the mothers are partially prevented from producing viable offspring, and are therefore partially physiologically isolated. As in the cross between *D. simulans* and *D. melanogaster*, so here in outcrosses of the lethal to non-lethal lines, the female offspring are rarely able to develop if the egg cytoplasm has been supplied by one of the lines, but develop readily if the cytoplasm has been derived from the other line (*i.e.*, in the reciprocal cross). The present case differs from the species cross in that it is possible to analyze the genetic basis of the cytoplasmic influence (which is not possible in the species cross because the hybrids are sterile), and in that evidence exists concerning the origin of this genetic basis by mutation from the parent stock. We have here, then, a condition approaching in some respects reproductive incompatibility, and this condition is dependent upon a specific genetic constitution. The suggestion is not offered that the lethal exhibits phenomena of complete physiological isolation, but that certain analogies to such isolation are shown in its behavior which may be of value in considerations of the genetic basis for the reproductive incompatibilities presented by related species and in determining how such incompatibilities arise.

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MUTANT FORMS OF MATTHIOLA RESULTING FROM NON-DISJUNCTION[1] [2]

FOUR of the mutant forms of stock described by Frost[3] (Large-leaved, Smooth-leaved, Crenate-leaved and Slender) have always produced, when selfed, a mixture of normal and mutant-type progeny. Three of these forms, Large, Crenate and Slender,

[1] Paper No. 116, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Cytological work done mainly by the junior author, in the Division of Genetics at Berkeley.

[3] Frost, Howard B. "Mutation in Matthiola." Univ. California Publ. Agric. Sci. 2: 81–190. 1919.

have recently been found to possess an extra chromosome in addition to the 7 pairs normal to the variety from which they arose. Occasional non-disjunction has been observed in the normal form, and this doubtless accounts for the origin of gametes with 8 chromosomes. Union of one of these with a normal gamete would produce a 15-chromosome mutant. About 2 to 5 per cent. of the progeny of normal plants have been classed as mutants.

When Slender and Large are intercrossed, four classes appear in their progeny—Normal, Large, Slender and Large Slender. Pollen mother cells of Large Slender at the first metaphase contain 9 chromosomes (7 bivalents and 2 univalents), and the root-tip cells contain 16 chromosomes. While it can not be said, on the basis of the observations now available, that the two odd chromosomes of Large Slender never pair, they certainly do so rather infrequently if at all. If Large and Slender were due to ordinary gene differences combined with trisomy in the same chromosome group, their normal progeny should be different. Since their normal progeny seem to be identical, it is probable that at least these two of the mutant forms are due to non-disjunction of chromosomes belonging to different normal pairs. This conclusion is strengthened by the great somatic dissimilarity of the two types; and in view of the situation presented in the final paragraph below, this further evidence is needed. Large Crenate also has two odd chromosomes; one of these resembles the odd chromosome of Crenate in form, and is unlike those of Large and Slender.

Plants of these four mutant types are generally more or less deficient in vigor as compared with normal plants, and statistical evidence of selective elimination of the former at germination has been secured.[3] There is also evidence of selective elimination of gametes carrying an extra chromosome, somewhat as in the case of simple trisomic forms in other genera. In Datura, Blakeslee[4] (summary) reports that the pollen of the trisomic Globe type has transmitted the Globe character to only about 2 per cent. of the progeny in back crosses to normal, while the ovules have transmitted it to about 26 per cent. In the 15-chromosome Oenotheras, de Vries and Boedijn[5] report that the tri-

[4] Blakeslee, Albert F. "The Globe mutant in the jimson-weed (*Datura Stramonium*)." *Genetics*, 6: 241–264. 1921.

[5] De Vries, Hugo, and Boedijn, K. "On the distribution of mutant characters among the chromosomes of *Oenothera Lamarckiana*." *Genetics*, 8: 233–238.

somic types are not reproduced at all by means of their pollen. Crenate Matthiola[3] is transmitted by the pollen to some 5 per cent. of the progeny, or about one fifth of the total percentage to which it is transmitted in selfing. Slender Matthiola differs decidedly from all these forms, since in back crosses the pollen has transmitted the mutant type to nearly one fifth of the progeny, and the ovules to about one third of the progeny, while the proportion of Slender from selfing is slightly higher still. In general, the four mutant types of Matthiola mentioned above have been transmitted, in selfing, to from 25 to 50 per cent. of the progeny; and it is plain that differential viability of both gametes and zygotes has contributed to this result.

It may be assumed that tetrasomic zygotes are usually nonviable. As with Globe Datura,[6] however, trisomic Slender parents give occasional progeny which manifest the mutant characteristics in an extreme degree. These Extreme-slender plants are very small and feeble. Some of their pollen mother cells contain 8 pairs of chromosomes; others, 7 pairs and 2 unpaired chromosomes. Cells with 8 pairs reduce normally, but in those containing 2 univalents the unpaired elements, like the single extra element in the 15-chromosome plants, divide in the first division and assort at random in the second. The very meager breeding data from one supposedly Extreme-slender parent[3] agree with the cytological results in that some normal progeny occurred, with an excess of Slender. There is no evidence that the odd chromosome of either Large or Slender unites characteristically with any of the 7 pairs of chromosomes.

The Slender type is associated with singleness of flowers, in a manner suggestive of linkage. The double-flowering plants, which are always completely sterile, are evidently pure recessives (*dd*).[7] The singles of a double-producing race are then *Dd*, and carry a pollen lethal which sterilizes all *D* pollen: selfed normal single parents usually give slightly more than 50 per cent. of double progeny. Slender gives a large excess of singles among its Slender progeny, and (except in the cross normal ♀ x Slender ♂) a large excess of doubles among its normal progeny. Slender as seed parent, whether selfed or pollinated by normal, gives an abnormally high proportion of total doubles

[6] Blakeslee, Albert F. "Variations in Datura due to changes in chromosome number." AM. NAT. 56: 16–31. 1922.

[7] Frost, Howard B. "The inheritance of doubleness in Matthiola and Petunia. I. The hypotheses." AM. NAT. 49: 623–636. 1915.

(about two thirds), which is presumably due to selective elimination of Slender. The general trend of the observed ratios is explained if we assume that all trisomic Slender singles adequately tested have been *Ddd* (*D* being dominant over *dd*), and that much selective elimination of the Slender type occurs. The breeding data, however, show a marked deficiency in the normal single class when Slender is seed parent, and the ratios are otherwise different from those expected. The basic gametic ratio from random reduction would be *2Dd* : *1dd* : *1D* : *2d*, the *D* pollen being non-functional as usual. For pollination of Slender by normal (pollen all *d*) the expected ratio (without selective elimination) is, therefore, 2 Slender single : 1 Slender double : 1 normal single : 2 normal double. The observed ratio is at present 56 : 21 : 22 : 137, and larger numbers from selfing show a similar departure from the expected ratio among the normals. The situation is evidently complex, and further consideration of hypotheses must be reserved for a more detailed report of this work to appear elsewhere.

A plant has been described[8] which had several Slender single branches, while the other branches and the upper main axis were normal double; if this plant was originally *Ddd* (Slender single), the bud variation is readily explained by early loss of the *D* chromosome in a cell of the apical meristem.

The fact that Crenate also shows genetic association with single[3] seems to be a serious difficulty in the way of any consistent and plausible general scheme for these forms. It is suggestive of the "varieties" of some of the trisomic forms of Datura,[6] although Slender and Crenate are not very similar morphologically.

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LUTEAL CELLS AND SEXUAL DIMORPHISM OF FEATHERING IN WILD BIRDS

BORING and Morgan (1918)[1] have shown that hen-feathering in the male Sebright Bantam is associated with the presence of

[8] Frost, Howard B. "An apparent case of somatic segregation involving two linked factors." AM. NAT. 55: 461–464. 1921.

[1] Boring, A. M., and Morgan, T. H., 1918, "Lutear cells and hen-feathering." *Journ. Gen. Physiol.* I.

luteal cells in the testis. Since luteal cells are also a constant element in the ovary of the domestic fowl it has been suggested that hen-feathering in female fowls and in the male Sebright is due to a hormone secreted by the luteal cells which suppresses the development of cock feathers.

These conclusions suggested an investigation of the question whether or not there is a correlation between the sexual dimorphism in the feathering of wild birds and the presence or absence of luteal cells in the gonads, and also an investigation of the relation of the structure of the gonads to the seasonal variation of plumage. It seems advisable to give at this time a brief statement concerning the first question, inasmuch as considerable time will be required to satisfactorily work out the latter problem.

In order to test out the possible relation of the presence of luteal cells to feather coloring, a histological study has been made of the gonads of both sexes of a number of wild birds which for convenience have been grouped as follows: (1) Those in which the female shows the higher degree of coloration, such as the northern phalarope; (2) those in which there is no marked difference in the coloring of the two sexes, such as the killdeer, spotted sandpiper, steller jay and water ouzel; and (3) those with the male possessing the more brilliant plumage, represented by the robin, western bluebird, flicker, bob white, California quail, rusty blackbird and China pheasant.

The observations recorded here were made on the gonads of birds taken in late winter, spring and early summer. Immediately after the birds were shot their reproductive organs were removed and put into Bouin's fluid. The sections were for the most part stained with Delafield's haematoxylin.

In an earlier report[2] it was shown that as regards the phalarope no evidence was obtained indicating that luteal cells in any way influence the difference in feathering in the sexes. In no case were any luteal cells found in the testes of the phalarope, while sections of the ovaries showed them in considerable abundance. Since, in this instance, the female is the more brilliantly colored, the evidence that luteal cells secrete a hormone having a suppressing influence on feather-coloring is negative.

A study of the gonads of the birds in the second group showed luteal cells present in all the ovaries, but with the possible excep-

[2] Yocom, H. B., "Luteal cells in the gonad of the phalarope," *Biol. Bull.* 44, March, 1924.

tion of the testes of spotted sandpiper no cells were found in the testes which at all resembled luteal cells. In the testes of the sandpiper there were large cells located between the seminiferous tubules, resembling somewhat the characteristically grouped luteal cells of the ovary. Whether or not these are actually luteal cells must remain undetermined until another migration period, when more material will be available.

In the gonads of the birds of the third group every ovary studied possessed characteristic packets of luteal cells, but in no case were any such cells found in the testes.

Of the birds thus far studied there is no positive indication that luteal cells are present in the testes, while in all cases they were found in the ovaries. Such evidence would indicate that, for the birds studied, it is not a hormone secreted by luteal cells that has a suppressing influence on the development of color in the feather. Indeed, no evidence is at hand which would warrant any suggestion concerning the function of these characteristic groups of cells in the ovaries of wild birds. Nonidez[3] has shown that in the fowls studied by him such cells arise from embryonic sex cords. This, however, does not give us any clue to their function, much less furnish evidence of their controlling influence on the color of feathers.

We must, however, bear in mind the fact, as suggested in the work on the phalarope, that in most fowls feather structure differs in the two sexes, while in the wild birds studied, with the exception of the gallinaceous birds, sexual differences in feathering seem to be due to color rather than to morphological differences of the feathers.

It is possible that a study of the relation of the structure of the gonad to the seasonal variation in feathering may give some evidence leading to an understanding of this perplexing question of the sexual dimorphism of feather color in wild birds.

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[3] Nonidez, J. F. 1922, "Studies on the gonads of fowls. The origin of the so-called luteal cells in the testis of hen-feathered cocks." *Am. Jour. Anat.*, 31, 109–124, 7 figs. in text.

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